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Foreword

We take this opportunity to thank all of the participants who together accounted for the success of the Eighth Annual Meeting of the Foundation. We are particularly grateful to the speakers whose contributions appear in this volume, and to Dr. W. H. Sebrell, Jr. and Dr. H. D. Kruse who so ably chaired the morning and afternoon scientific sessions, respectively.

Robert S. Goodhart, M. D.
Editor, Nutrition Symposium Series

NEWER KNOWLEDGE OF THE METABOLISM OF TOCOPHEROLS IN HUMAN TISSUES*†

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Vitamin E, or alpha tocopherol, represents one of the great enigmas of human nutrition. During the quarter century or more since its discovery, it has become recognized as an essential nutrient for more than 20 species of laboratory and farm animals. Furthermore, deficiency manifestations experimentally induced have counterparts in disorders occurring spontaneously in domestic animals under field conditions. Dystrophic lesions of the skeletal musculature are common to all forms studied; other lesions or dysfunctions, involving cardiac muscle, smooth muscle, the reproductive systems and the vascular system, show considerable species variability. These manifestations may reflect a lack of regulatory control which alpha tocopherol exerts over oxidative mechanisms of the cell, but we are ignorant of the metabolic mechanisms involved and not certain whether this represents the only function of tocopherol.

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* The data presented are based largely upon a thesis of the junior author (M Y D.) submitted to the Graduate Faculty of the University of Rochester in partial fulfillment of the requirements of the degree of Doctor of Philosophy, 1953.

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Most vitamins, including the other fat-soluble vitamins (A, D and K), have bridged with considerable speed and equanimity that hazardous gap between experimental exploration and practical application in clinical medicine. Such has not been the case with Vitamin E, despite established parallels between experimental findings and experiences in veterinary medicine. We not only lack evidence of a syndrome of avitaminosis E in man, but are faced with the dilemma that dysfunctions in man which represent the closest counterparts of those seen experimentally are generally unresponsive to tocopherol therapy, whereas many clinical states quite dissimilar to those experimentally induced are said to be benefited by tocopherol therapy, but usually at levels which are more pharmacologic than physiologic.

Tocopherols are widely distributed in human foods, but rarely present in high concentration. On a good dietary the average daily intake of alpha tocopherol is about 25 mg. Plasma tocopherols, in health and disease, range from 0.5 to 1.5 mg/100 cc. A mean of about 1.2 mg/100 cc for healthy adults is generally accepted. Published data relative to tissue tocopherols in man, restricted to a few cases of accidental death, have clearly indicated the need for more information of this type in order to obtain some picture of the normal tocopherol status of the human organism as a whole, and the manner in which this may be influenced by disease states of one type or another. It is for these reasons that we have endeavored to explore the distribution of tocopherol in human tissues from intrauterine life to advanced old age, selecting for the most part material from cases of death due to accident or illness of short duration, in the hope of establishing a reasonably adequate norm.

The macrochemical procedure developed by Quaife and Dju[1] for measuring tissue tocopherols, and applied by them to the analysis of tissues from two cases of accidental death, has been employed in the analysis of more than 1100 tissue samples over the past 3 years. Tissues were stored in deep-freeze and usually analyzed within 2 to 3 weeks of acquisition. When shipped from outside sources, they were kept frozen with dry-ice. Each analysis represents, on the average, approximately one half-day's effort. Values were routinely obtained for total lipid, for total tocopherols and for gamma and delta tocopherols combined. Alpha tocopherol was the predominant, and often the only type found. In the data presented here, the results are expressed in terms of milligrams of total tocopherols per 100 grams of fresh tissue, and also as milligrams per gram of extractable fat.

I. Tocopherol Status Prior to and at Birth

The record of clinical experiences relating to other fat-soluble vitamins, together with accumulated evidence of rather limited transfer of tocopherol across the human placenta (marked differences noted between tocopherol levels of maternal and cord blood, and a few reported analyses indicating lower levels of tocopherol in the fetus and newborn than in the placenta), directed our attention first to the tocopherol status of man during prenatal and early postnatal life. This phase of our study is based upon analyses of 23 human fetuses at 2-6 months gestation age (chiefly therapeutic abortions) and 15 of the corresponding placentae, 12 term placentae, and a variety of separate tissues and organs from 9 fetuses and from 18 premature and 6 full term infants which succumbed at or within a few days of birth.

Table 1

Tocopherol Levels in Fetuses (2-6 months) and Placentae

Material	Lipids %	Total Tocopherols (average) mg/100 g fresh tissue	mg/gm fat
23 Whole Fetuses	1.42	0.32	0.26
15 Placentae (of same)	1.57	0.71	0.49
12 Placentae (term)	1.51	0.50	0.32
Skeletal Muscle (9 fetuses)	1.08	0.59	0.57
Liver (7 fetuses)	2.26	0.57	0.27

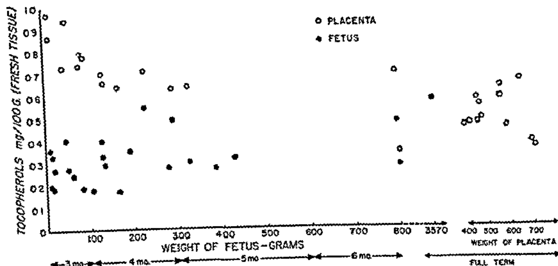


FIGURE 1. *Scatter-graph showing tocopherols, as mg %, in 23 fetuses of 3 to 6 months gestation age and 15 of the corresponding placentae; in one full term infant of 3570 gm analyzed in toto; and in 12 full-term placentae.*

Our data, presented in more detail elsewhere[2], indicate that the fetus and newborn infant are not overly endowed with tocopherol (Table 1). Tocopherol levels in whole fetuses of 2 to 6 months gestation age were consistently low, usually less than one-half those of the corresponding placenta. Placental levels were somewhat lower at term than at or before the sixth month (Fig. 1). However, the increased tocopherol levels found in the full term stillbirth analyzed in toto (Table 2), and comparisons between individual tissues of fetuses and of premature

infants, suggest some increment in fetal tocopherols during late pregnancy. This is in accord with our observations[2] that premature infants of increasingly greater body weights (and presumably representative of fetuses during the last trimester of pregnancy) show a tendency for tocopherol levels to increase slightly in most tissues, and somewhat more so in the adipose tissue which first makes its appearance at the 6th to 7th month. Considering average values for tissues and organs of the 18 prematures and 6 term infants, it will be noted (Table 2) that the adipose tissue, although embryonic in type and often meager in amount. possessed the highest levels; the next in order were liver, heart and skeletal muscle; the values for lung, kidney and intestine were similar to, or slightly lower than, those for muscle.

Table 2
Tocopherol Levels in Newborn Infants

	Total Tocopherols (average)			
	18 Prematures	6 Term*	24 Infants Combined	
Tissue	mg %	mg %	mg %	mg/gm fat
Adipose Tissue	2.09	2.40	2.14	0.07
Liver	1.06	0.70	0.97	0.28
Heart	0.84	0.55	0.76	0.35
Skeletal Muscle	0.66	0.42	0.61	0.30
Lung	0.62	0.74	0.65	0.26
Kidney	0 62	0.56	0.60	0.24
Intestine	0.57	0.69	0.60	0.32

* These include one stillbirth which, after selection of tissue samples for analysis, was analyzed in toto. Values of 0 56 mg %, and of approximately 20 mg for the entire infant, were obtained.

At this point, reference should be made to a well recognized natural increment of about 65% in plasma tocopherol levels during the latter part of pregnancy[3]. The reason for this is not clear; it may represent an effort to overcome restricted placental transfer, or it may reflect a physiologic preparation for more effective transfer of

tocopherols to the milk to better prepare the infant for the exigencies of early extrauterine existence. Human breast milk has a considerably higher tocopherol content than cow's milk; furthermore, in both species colostrum is much richer in tocopherol than is later milk[4].

It might also be mentioned that the premature and term infants used in this study are representative of the great majority of infants in which, at necropsy, respiratory failure or embryologic anomalies appear to be the primary *causes of exitus. Since it seems unlikely that their inability* to survive was in any way related to their tocopherol status, the latter should provide a reasonably normal picture of tocopherol levels at the dawn of life in man. It is not too bright a picture when we note that the total tocopherol content of the full term stillbirth, analyzed in toto, was only about 20 mg., or approximately the daily *intake of adult man on a high-quality diet.*

II. Tocopherols in Infancy

What happens after birth is still another story. Let us consider first the bottle-fed premature infant. Denied the benefit of placental transfer of tocopherol during the latter phases of gestation, physiologically handicapped in *his postnatal adaptations to his new environment*, and usually given a low-fat formula providing about one-fifth the tocopherol content of breast milk, such infants at birth possess serum tocopherol levels about one-fifth those of the mother and, according to our data, a rather low tissue storage. During the first month of postnatal existence, as *shown by Wright, Filer and Mason*[5], *he manifests a* diminishing serum tocopherol level which may closely approach that which in the young experimental animal produces typical vitamin E deficiency symptoms. On the other hand, the bottle-fed full term infant, with similar initially

low serum levels but reared on a less restricted formula, shows a small but gradual increment in plasma tocopherol; whereas the breast-fed infant, even though not benefiting from the unusually high tocopherol content of colostrum, exhibits a rather remarkable postnatal increment in plasma tocopherols.

There are other, and rather interesting, reasons for believing that the human infant at birth has critically low tocopherol storage. Dr. Paul György and his associates[6] have found that the red blood cells of term infants, during the first week or so of postnatal life, show mild hemolysis when exposed to low concentrations of hydrogen peroxide; a similar reaction of erythrocytes to hydrogen peroxide and to dialuric acid is shown by newborn stock rats and by adult rats low in vitamin E[7]. We have observed a similar phenomenon in the vitamin E deficient monkey. Gordon and deMetry[8] find the fragility test positive in bottle-fed premature infants for a month or more after birth, but negative within 2 to 5 days after giving tocopherol to the infant, as observed by György *et al.*[6] in full term infants. Oral administration of moderate doses of tocopherols to mothers during the last few weeks of pregnancy seems not to overcome this physiologic tocopherol deficiency of the newborn infant[6], due presumably to negligible placental transfer of tocopherols in a biologically available form. The observations just mentioned when considered in relation to the low tissue levels observed in infants at birth and during the first three years of postnatal life (Table 3), and to extensive experimental evidence that manifestations of vitamin E deficiency are much more readily induced in infantile than in older animals, raise the question of whether pediatricians should not give careful consideration to the matter of tocopherol supplementation of infants, especially bottle-fed infants.

Table 3

Tocopherols in Human Tissues—Birth to Old Age

Materials / Age	No Cases	Total Tocopherols (average): Muscle mg %	Muscle mg/gm fat	Heart mg %	Heart mg/gm fat	Liver mg %	Liver mg/gm fat	Adipose Tissue mg %	Adipose Tissue mg/gm fat
Newborn	24	0.61	0.30	0.76	0.35	0.97	0 28	2.14	0 07
3 da-3 yrs [a]	14	0 44	0 20	0.43	0 14	0 82	0 20	1.65	0 04
	6	0 60	0.30	1 00	0 37	0 82	0 21	3.21	0.07
4-10 yrs.[b]	6	1.07	0.40	1 33	0 43	1 52	0.41	5.21	0 11
12-18 yrs [c]	12	0 97	0.31	1.03	0 36	1.48	0.33	9.11	0 20
23-52 yrs.[d]	20	1.24	0 38	1 15	0.33	2.08	0.35	8 31	0.12
61-93 yrs [e]	10	0.93	0 14	1 07	0 30	0 89	0.26	6 09	0 10

[a] This group has been divided arbitrarily into 14 cases of death due chiefly to diseases of the liver, kidney and lung, and 6 cases of congenital heart disease or accidental death.

[b] Four deaths due to auto accidents, one to burns, and one to drowning.

[c] Five deaths due to accidents, 7 to relatively acute disease processes

[illegible] disease, 5 of chronic alcoholism, and 7 [illegible] groups were not significantly different, [illegible] and decreased levels in adipose tissue

[e] All represent cases of cardiovascular disease.

Our data on normal tissues representing the first few years of postnatal life are limited, due to the relative infrequence of necropsies on cases of sudden or accidental death in this age group. Tissue tocopherols in 14 infants in which diseases of the liver, kidney and lung predominated are appreciably below those of newborn infants. These findings are in accord with other observations[9] that plasma tocopherol levels are often quite low in infants suffering from such disorders. Tissue tocopherols in the 6 cases of accidental death in this age group are similar to those of newborn infants, suggesting that increased growth and other metabolic demands of early postnatal life may normally result in utilization of tocopherols at a rate such that little or no tissue increment can occur. On the other hand, the 6 cases of accidental death in the 4 to 10-year age group show considerably higher tissue tocopherol levels which, except for adipose tissue, are quite comparable to those found in later life. These differences may reflect changes occurring in both tocopherol and lipid content of tissues during early childhood.

III. Tocopherols from Childhood to Old Age

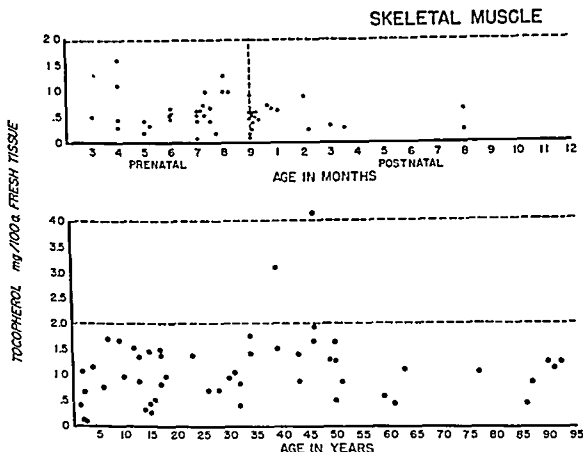


FIGURE 2. *Scatter-graph of values for total tocopherols, as mg %, in skeletal muscle from fetuses of 3 to 6 months, prematures, full term infants, and infants up to 8 months of age (upper portion) and from children, adolescents and adults varying in age from 1 to 93 years (lower portion). The broken lines at the levels of 2 mg % and 4 mg % are arbitrary ones for convenience in comparing values with those recorded in the following three scatter-graphs, and with the accepted normal range of plasma tocopherols for man (0.05 to 1.5 mg %) which would occupy the central half of the area under the broken line at 2 mg %. It is of interest that tocopherol levels for muscle fall within the 0-2 mg % area, except for two values recorded and two values of 5.48 (a case of progressive muscular dystrophy) and 6.58 (a case of metastatic carcinoma) which exceeded the limits of the graph.*

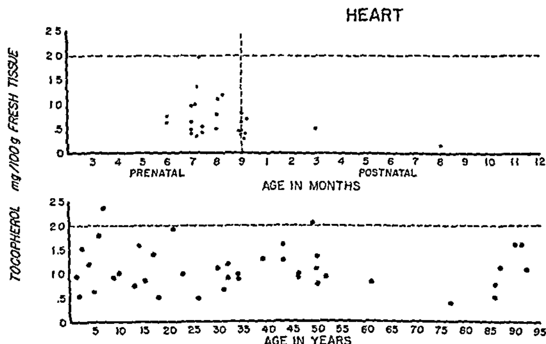


FIGURE 3. Scatter-graph of values for total tocopherols in cardiac muscle, recorded on the same basis as those for skeletal muscle in figure 2; samples from early fetuses were too small for chemical analyses. Here also, all values except two fall within the area under the broken line at 2 mg %.

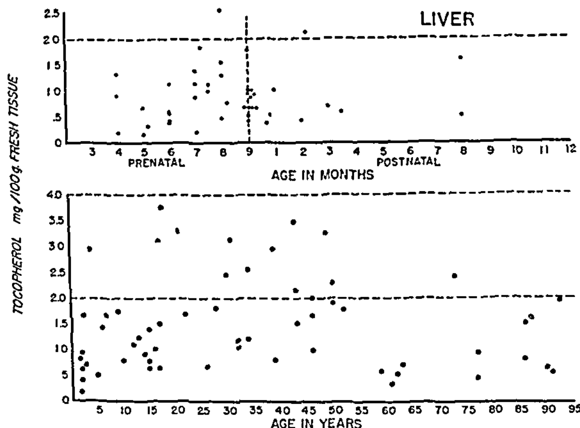


FIGURE 4. *Scatter-graph of values for total tocopherols, in the liver at various ages, recorded on the same basis as the values for skeletal and cardiac muscle in figures 2 and 3. Those values above the broken line at 2 mg % in the lower portion of the graph represented chiefly cases of accidental death or of chronic alcoholism; one value of 5.36 mg % (chronic alcoholic) exceeded the limits of the graph.*

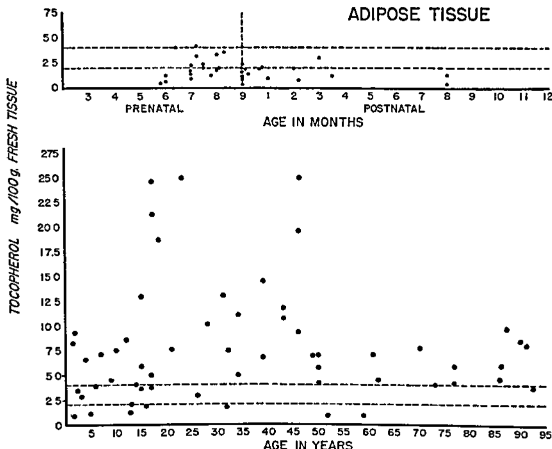


FIGURE 5 *Scatter-graph of values for total tocopherols in adipose tissue at different ages. Because of the much higher values, the units of the ordinate have been reduced to one fifth those of the three preceding graphs; even then, one value of 43 mg % (case of myasthenia gravis) could not be included. Attention should be called to the fact that when tocopherol levels in adipose tissue were expressed in terms of mg/gm of extractible fat, the values were of a lower order than for skeletal muscle, heart or liver.*

Four tissues have been routinely analyzed, when obtainable—skeletal muscle (psoas and pectoralis, mixed), heart, liver and adipose tissue (subcutaneous and retroperitoneal, mixed)—selected because they can usually be secured readily and in desired amounts (5-10 grams) at necropsy, and

together represent more than one-half the total body mass. Tissue tocopherol values obtained are recorded individually in the form of scatter-graphs (Figs. 2-5) to indicate variations and age relationships, and as averages for different age groups (Table 3). The results will be discussed first from the standpoint of tocopherols, as mg %, in the different tissues studied. This provides a ready means of correlating values reported for blood, which are customarily expressed in the same manner.

Skeletal muscle. It will be noted that beginning with the 4 to 10-year age period, muscle values are appreciably higher than at earlier ages and tend to remain at much the same level throughout the rest of the life span. It is of particular interest that, with but few exceptions, values fall below the broken line indicating levels of 2 mg % (Fig. 2); furthermore, 62% of them come within, and 19% above, the accepted normal range of plasma tocopherol levels (0.5-1.5 mg %).

Heart. Tocopherol levels in heart muscle at various age periods are not significantly different from those of skeletal muscle, and warrant no particular comment. About 73% of the values in the lower part of figure 3 fall within, and 22% above, the 0.5-1.5 mg % range.

Liver. From fetal life to late middle-life tocopherol values for liver tend to be somewhat higher than for skeletal or cardiac muscle (Fig. 4; Table 3); about 49% are within the normal plasma tocopherol range, and 44% are considerably higher than this. The lower values in the older age group (61-93 years), composed chiefly of cases of arteriosclerotic heart disease and of coronary and cerebral thrombosis, may be of some metabolic significance. So also may be the fact that of the 13 values exceeding 2 mg % (Fig. 4)[10], represent cases of death from automobile

accidents, homicide, or chronic alcoholism, or combinations of the three. It is tempting to speculate that the higher levels may be more representative of normal individuals and that acute illnesses, such as characterized most cases from which the other liver specimens were obtained, result in a depression of normal liver tocopherols.

Adipose tissue. Beginning with its first appearance in the fetus at the 6th to 7th month, adipose tissue regularly shows a much greater concentration of tocopherols than the other tissues, per unit weight of fresh tissue. It will be noted that the ordinate units of the scatter-graph (Fig. 5) have been increased five-fold in order to accommodate the relatively high values. The increase in these values during childhood and adolescence and the diminution late in life (Fig. 5; Table 3) are related in part to changes in extractible lipids and in part to changes in tocopherol concentration per unit of lipid.

Consideration of results expressed as mg per 100 g fresh tissue, such as employed in the scatter-graphs and the left hand columns in Table 3, give the impression of a rather abrupt increment in tissue tocopherols at 4 to 10 years of age followed by no very significant changes for the next 50 years, except in adipose tissue which appears to become, with increasing age, somewhat of a storage depot for tocopherols. However, when results are expressed as tocopherols per gram of extractible fat, there appears to be relatively little variation in the average values for muscle, heart and liver, irrespective of age, except for low values in the group of 14 infants of the 3-day to 3-year group where conditions impairing fat absorption were common causes of death, and low values for skeletal muscle in the old-age group. It is also noteworthy that on this basis the values for adipose tissue in each age group are consistently much lower than those for the other tissues (Table 3).

The lipids of muscle, heart and liver are considered to be metabolically more active and richer in unsaturated fatty acids and phospholipids than are those of adipose tissue. The concentration of tocopherol in the tissues studied is within the range at which other stabilizing agents or antioxidants might be expected to operate effectively. For these reasons, coupled with the evidence that the contentration of tocopherol in the lipids of muscle and liver is relatively constant and appreciably higher than that of adipose tissue, our findings appear to be quite compatible with the concept that a primary function of tocopherol is that of a vital intracellular antioxidant.

Table 4

Tocopherol Levels in Endocrine Glands and Other Visceral Organs

Material		Lipids %	Total Tocopherols (Average) mg %	mg/gm fat
18 Adrenal	21-77 years	26.00	13.17	0.73
22 Pituitary	26-50 years	3.62	4.04	1.24
11 Testis	21-50 years	3.78	4.01	1.01
5 Ovary	32-50 years	1.65	1.10	0.62
19 Pancreas	23-91 years	8.05	1.81	0.31
5 Uterus	28-45 years	1.44	0.85	0.72
10 Spleen	12-90 years	2.44	0.81	0.34
7 Kidney	17-90 years	3.00	0.68	0.28
4 Lung	61-91 years	1.98	0.37	0.19

Other tissues. The data recorded in Table 4 indicate that, compared to values obtained for muscle, heart and liver (Table 3), tocopherols as mg % were low in lung, kidney, spleen and uterus, of about the same order in pancreas and ovary, much higher in testis and pituitary, and exceptionally high in adrenal where values exceed those for adipose tissue. In terms of tocopherols per gram of fat, the

pituitary ranked highest of all tissues studied, with the testis and adrenal next in order. These data raise interesting questions relative to possible functions of tocopherols apart from those of intracellular antioxidants.

Progressive Muscular Dystrophy. In the course of these studies we were able to secure samples of 8 separate muscles and of 8 other tissues and organs from a 21-year old male with advanced progressive muscular dystrophy who succumbed to bilateral bronchopneumonia after a brief illness. Muscles representative of the neck, thorax, abdomen and pelvic girdle showed, in that general sequence, an increasing severity of degenerative change and fatty replacement, an increasing content of lipid (4.41-71.77%; aver. 32.67%) and of tocopherols (2.22-10.51 mg %; aver. 5.48 mg %), and a diminishing concentration of tocopherols per unit of fat (0.50-0.07 mg/gm fat). Tocopherols were moderately low in adipose tissue (7.70 mg %; 0.09 mg/gm fat) but quite normal in the heart, liver and other organs. These data, and those from analyses of biopsy specimens of muscle from 8 patients with progressive muscular dystrophy, extend the evidence, previously based only on plasma tocopherol studies, that in muscular dystrophy there is no general or local tissue inadequacy of tocopherols. That there may exist a metabolic defect in the capacity of skeletal muscle to convert the tocopherol present into some other compound (such as tocopherylhydroquinone), as suggested by Milhorat *et al.*[10], represents a likely possibility and one on which attention is being focussed at the present time.

Discussion

Although we know but little regarding the metabolic functions of vitamin E and are unaware of any state of true

avitaminosis E in adult man, we now have a more complete picture of its distribution throughout human tissues at all ages than we have of any other vitamin. The wide distribution of tocopherols in various tissues, usually at concentrations not greatly different from those in blood, and the absence of any true storage depot such as the liver provides for vitamin A, are quite comparable to what is known concerning tocopherols in lower animals. Our analyses of tissues from normal rhesus monkeys have indicated a pattern of tocopherol distribution which essentially duplicates that seen in man; moreover, tissues from monkeys reared on low E diets to the point of showing negligible or mild dystrophic lesions of the skeletal muscles, have revealed tocopherol levels approximating those observed in the human fetus.

The close correlation seen between tocopherols and lipid in the fetus[2], and that noted in tissues and organs at later ages in the studies presented here, clearly points to an important tissue-antioxidant function of tocopherols in man. This, of course, does not exclude the possibility of other more specific functions of which we are as yet unaware. The question of whether man ever reaches a state of tocopherol tissue depletion which is reflected in any specific deficiency manifestation or histopathologic change can be answered in part. Certainly such a state is approached in the infant, especially the premature infant, at birth and during the first few weeks of postnatal life. Conceivably, conditions of chronic absorptive defects and metabolic stress in later life could lead to similar degrees of tocopherol depletion. The analyses which we have carried out have been directed toward establishing a set of reasonably normal values which, it is hoped, may subsequently provide a better means of evaluating instances of suspected inadequacy of vitamin E even though the criteria employed must depend upon biopsy or necropsy material.

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Summary

The data presented in this report demonstrate that tocopherols, predominantly alpha tocopherol, are widely distributed in human tissues from early fetal life to advanced old age. Tocopherol levels, expressed as mg/100 gm of fresh tissue, are low in fetuses of two to six months gestation age, and only slightly higher in premature and full term infants at birth. (One stillbirth, analyzed in toto, contained about as much tocopherol as the daily intake of an adult.) Data presented are in accord with other evidence that during the early postnatal period of life states of suboptimal vitamin E nutriture may occur. During early postnatal life, tissue levels tend to increase slowly unless suppressed by diseases of various types. During childhood and adolescence they reach levels comparable to those of adults which, for muscle, heart, liver and certain other visceral organs are approximately twice those at birth, and for adipose tissue are considerably higher than in other tissues, except the adrenal. During advanced age there is a tendency for tocopherols to decrease in liver and adipose tissue.

Tocopherol levels, expressed as mg/gm extractable fat, are maintained within a rather limited range in most tissues at all ages, but are relatively low in adipose tissue and particularly high in the pituitary, testis and adrenal.

In progressive muscular dystrophy tocopherol levels are relatively high in the skeletal muscles, and within the normal range in other tissues and organs.

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STUDIES ON THE FUNCTIONS OF VITAMIN B_6*

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Vitamin B_6 has been implicated in the metabolism of both fat and protein. In the latter case, interest has centered about the vitamin as the coenzyme for several transaminases and for amino acid decarboxylases. There is little evidence regarding the metabolic purposes served by these enzymes in an intact animal. This communication is a summary of studies designed to furnish information regarding the functions of vitamin B_6 in the rat. One experimental result has had an interesting application to investigations on humans and it is hoped that other observations will be similarly useful.

It is well known that young rats, deprived of a dietary source of vitamin B_6, fail to increase body weight and that there develops a marked difference in body weight between the deficient animal and an *ad libitum* control. Pair-feeding has been used for some years to provide a control with a food intake similar to that of the deficient animal. It has been observed repeatedly in studies on vitamin B_6 that the pair-fed control has, after several weeks of experimental feeding, a body weight intermediate between the body weight of the *ad libitum* control and that of the deficient rat, but significantly greater than the latter.

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This observation is so simple and so well known that it has been overlooked in interpreting more complicated studies. It should not be brushed aside because it may serve as the fundamental observation to elucidate the physiological effects of vitamin B_6.

It is only possible for the pair-fed control to become significantly heavier than the deficient animal by having available for body increment food constituents in excess of the amount needed to meet energy requirements and for tissue replacement. The deficient rat does not increase in body weight because it has no such surplus. Remembering that the food intake is the same for both animals, we have three possible explanations:

1. Absorption of food is less in the deficient animal and a smaller amount is available within the tissues.
2. The basal metabolic rate is increased in the deficient animal.
3. The efficiency of food utilization is decreased by a deficiency of vitamin B_6.

In 1951 Carter and Phizackerley[1] reported that vitamin B_6 deficiency does not impair the ability of the rat to absorb protein, fat, or carbohydrate. A similar observation regarding protein has been made in our laboratory[2]. It would seem that the difference in body weight between the deficient rat and the pair-fed control is not due to an alteration in absorption of foodstuffs.

It should be noted that the difference in body weight, with its attendant implications of changes in metabolism, is not, of course, specific for vitamin B_6. A similar difference has been observed repeatedly consequent to defi-

ciencies of other nutrients. In early experiments, in which pair-feeding was not employed, emphasis in interpretation was placed either on an effect on appetite or on an interference with growth.

The second possible explanation of the difference in body weight is that the vitamin deficiency causes an alteration in basal metabolic rate. This has been studied[3] on individual rats as the deficiency state progressed with coincident measurements on pair-fed and on *ad libitum* controls. Basal metabolic rate has been expressed in volumes of consumed oxygen per square meter of body surface per minute. On this basis the basal metabolic rate for *ad libitum* controls remained reasonably constant through four weeks. The metabolic rate of the pair-fed controls decreased progressively, probably because of inanition. The metabolic rate of deficient rats was about equal to that of the pair-fed controls and appeared to be unaffected by a deficiency of vitamin B_6. The difference in body weight cannot be explained as resulting from a change in metabolic rate caused by the vitamin deficiency.

In view of the difference in body weight it seemed advisable to examine the effect of vitamin B_6 deficiency on carcass composition. It had been observed previously[4] that vitamin B_6 deprivation in rats fed a high-casein diet devoid of fat and carbohydrate resulted in low carcass fat. This was interpreted, in conjunction with higher carcass fat found in pair-fed controls, as evidence that vitamin B_6 is necessary for the synthesis of fat from protein. In the recent studies[5] rats were maintained on a diet containing 20 percent casein and 74 percent sucrose. The carcass composition of deficient rats remained fairly constant throughout the experimental period. Pair-fed controls had a marked increase in the proportion of fat, a decreased pro-

portion of water and a fairly constant proportion of protein. The salient finding was that deficient animals, with the same food intake as the controls, were unable to increase body stores of fat. A similar result was obtained with other animals given a diet containing 20 percent corn oil replacing a like amount of sucrose. The difference in body fat between deficient rats and pair-fed controls is not specific for vitamin B_6; similar observations for thiamine deficiency have been made in several laboratories and recently for vitamin B_{12} by Ling and Chow[6]. Either all three vitamins, and perhaps others, are concerned with fat synthesis and metabolism or else these vitamins produce the effect indirectly through an aberration in energy production. It is interesting to note that guinea pigs deficient in ascorbic acid contain more carcass fat than do pair-fed controls[7], a reverse of the effect seen in the case of vitamin B_6 and other B vitamins.

Over some years there was considerable interest in a possible interrelation of vitamin B_6 and fat metabolism, particularly regarding essential fatty acids. In the present studies[8] the inclusion of 20 percent corn oil in the diet, which also contained desoxypyridoxine, did not prevent acrodynia but did render the lesion less severe.

Using isocaloric feeding of two basal diets, one high in casein, the other in carbohydrate, and both free of fat, alterations in liver and carcass lipids have been studied[9]. With both basal diets, the deficient rats had an increased proportion of unsaturated fatty acids in the carcass and an increased amount of phospholipids. The pair-fed controls contained, of course, a greater amount of fat in the carcass and this fat had an increased proportion of saturated acids. The liver fat of both groups was about equal in amount and in composition. There appears to be a conservation of

phospholipids and of unsaturated fatty acids in the rat in which vitamin B_6 deficiency is being developed.

One result of the studies on body composition should be noted. In the deficient rat body protein is kept at a fairly constant level. The replacement of amino acids in tissue proteins is not impaired. Deprivation of vitamin B_6 produces, also, no decrease in blood proteins.

The effect of vitamin B_6 on nitrogen balance has been studied[9]. Deficient rats exhibited an increased urinary excretion of nitrogen and a consequent decreased nitrogen retention. In no case was a negative nitrogen balance observed but deficient rats had a less positive balance than did pair-fed controls. The difference between deficient rats and pair-fed controls was statistically significant and was consistent with observations on body composition.

The increase in urinary nitrogen is compatible with the observation made in 1946[10] that vitamin B_6-deficient rats have an elevation in fasting blood urea. This finding has been confirmed repeatedly[11]. After the administration of test loads of urea, this substance disappears as quickly from the blood of deficient rats as from the blood of pair-fed controls. No evidence of renal failure has been obtained. Moreover, the rate of urea formation in slices from the livers of deficient rats has been found to be significantly greater than in slices from the livers of pair-fed controls[12]. There is now clear evidence that urea formation is not only not impaired by vitamin B_6 deficiency but is actually augmented. It is interesting that data from deficient rats have shown a statistically significant inverse relation between the rate of urea production in liver slices and the transamin-- activity of homogenates from the same livers.

Fasting levels of several metabolites in blood fr- cient rats have been compared with values for

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mals[14]. In both cases, growth hormone failed to produce the increased nitrogen retention which was manifest in *ad libitum* controls. The lack of this typical effect of growth hormone could be explained by pointing out that deficient rats have a lessened food intake and that insufficient food may be available in such animals to permit a more positive nitrogen balance. This explanation has been substantiated by the failure of growth hormone to increase either body weight or nitrogen retention in pair-fed controls. However, growth hormone did have certain effects on the deficient animals: increased severity of acrodynia, augmented elevation of the fasting level of blood urea, lowered content of total vitamin B_6 in the liver. It is notable that all of these consequences of growth hormone administration were essentially an aggravation of the deficient state. There may be doubt as to whether growth hormone brings about nitrogen retention by depressing amino acid catabolism or by augmenting the incorporation of amino acids into tissue proteins, but the net result is the reverse of the effect on protein metabolism produced by deficiency of vitamin B_6. The experimental evidence prompts this suggestion: in the vitamin B_6-deficient rat increased catabolism of amino acids is brought about to provide energy. Growth hormone tends to prevent this and, in doing so, accentuates the deficiency state.

At least one steroid hormone of the adrenal cortex is said to augment the catabolism of amino acids. In our laboratory[14] adrenalectomy did not prevent the metabolic changes of vitamin B_6 deficiency but was effective in preventing the development of acrodynia. The administration of ACTH and of adrenal cortical extract to rats maintained on a customary B_6-deficient diet did not alter, either positively or negatively, the pattern of deficiency. The administration of oestrogenic hormone was also without effect

controls[2]. Plasma glutamine is significantly decreased by vitamin B_6 deficiency. No significant differences were noted for blood amino nitrogen, serum protein, and plasma glutamic acid. The difference in plasma glutamine is interesting in view of the observations on the formation of urea.

The use of a test load of an amino acid in studies on vitamin B_6 deficiency was reported from our laboratory in 1946[10]. It was shown that blood urea remains at a high level in deficient rats for at least six hours longer than in pair-fed controls after a test load of alanine. This observation was utilized in tests of the effectiveness of pyridoxine in cases of hyperemesis gravidarum[18]. A similar effect on blood urea has been seen recently[11] in deficient rats given test loads of either lysine or glutamic acid. In all cases the rise in blood urea could not be explained as resulting from blood concentration. Reports on the relation of vitamin B_6 to the metabolism of tryptophane have given an impression that the vitamin is essential for the utilization of only this amino acid. There is now evidence that vitamin B_6 deficiency causes a distortion in the metabolism of at least six amino acids.

The increase in fasting blood urea, the prolonged rise in blood urea after a test load of an amino acid, the increased rate of urea formation in liver slices, the increased urinary nitrogen, and the existence of a less positive nitrogen balance are all consistent with a hypothesis that the catabolism of amino acids is augmented in the vitamin B_6-deficient rat. In view of this evidence it seemed desirable to study the effects of hormones which are believed to either increase or depress amino acid catabolism.

The effects of growth hormone on vitamin B_6-deficient rats have been studied on both young and adult ani-

replacement is not impaired in the deficient animal and, indeed, that protein can be synthesized to an extent limited by available supplies of amino acids. During the development of the deficiency, vitamin B_6 is mobilized from body tissues to the liver, presumably in an attempt to maintain hepatic enzymes in which the vitamin acts as a coenzyme. Basal metabolic rate, as expressed in volume of oxygen per minute per unit of body area, is the same in the deficient and pair-fed rats. In the light of this evidence it seems that the absence of excess food in the deficient rat to permit an increase in body weight results from a more wasteful use of foodstuffs in the provision of energy. This implies alterations in metabolism. The rate of urea formation in the liver is increased by the deficiency and the fasting level of blood urea is elevated. As would be expected, the deficient rat shows an increased output of nitrogen in the urine and is in a state of less positive nitrogen balance. It may be concluded that the catabolism of amino acids is increased. If we accept a frequently stated assumption that the main function of vitamin B_6 is exerted in transaminase systems, it could be concluded from the above evidence that transamination is not essential for the catabolism of amino acids nor for the production of urea.

The studies which have been reported have been carried out by a number of colleagues. The names of all of them appear in the bibliography and indebtedness is expressed to them.

Several years ago we reported[15] that the residual amount of total vitamin B_6 in the livers of acutely deficient rats was about two-thirds of the quantity in control tissues and was not altered by variation of the proportion of carbohydrate, fat, and protein in the basal diet. It seemed surprising that so much of the vitamin could remain in the livers of severely deficient animals. More recently it has been demonstrated[16] that vitamin B_6 is mobilized from the carcass to the liver as the deficiency progresses. A minimal, and possibly critical, level of the vitamin is attained about one week before acrodynia is manifest. It is suggested that the principal site of vitamin B_6 function is in the liver.

Except perhaps for the dermatitis, there have not been described structural changes in tissues as a consequence of vitamin B_6 deficiency. The primary lesion or lesions is, as Peters said in discussing thiamine deficiency, biochemical. There is substantial evidence relating vitamin B_6 to transaminase and decarboxylase systems. However, there is no clear picture of the metabolic derangement in an intact animal.

In the brief summary of recent studies there have been suggestions of the changes taking place in the rat deprived of vitamin B_6 and maintained on a diet containing 20 percent casein and 74 percent sucrose. Through about six weeks body weight remains fairly constant as does food intake. The pair-fed control exhibits a gradual increase in body weight. Carcass analysis has shown that the body composition of the deficient rat remains reasonably constant and that the pair-fed control is able to increase both fat and protein retention. Such an increase would be possible only if there were available an excess above the quantity of food necessary to meet requirements for energy and for tissue replacement. Present evidence indicates that tissue

replacement is not impaired in the deficient animal and, indeed, that protein can be synthesized to an extent limited by available supplies of amino acids. During the development of the deficiency, vitamin B_6 is mobilized from body tissues to the liver, presumably in an attempt to maintain hepatic enzymes in which the vitamin acts as a coenzyme. Basal metabolic rate, as expressed in volume of oxygen per minute per unit of body area, is the same in the deficient and pair-fed rats. In the light of this evidence it seems that the absence of excess food in the deficient rat to permit an increase in body weight results from a more wasteful use of foodstuffs in the provision of energy. This implies alterations in metabolism. The rate of urea formation in the liver is increased by the deficiency and the fasting level of blood urea is elevated. As would be expected, the deficient rat shows an increased output of nitrogen in the urine and is in a state of less positive nitrogen balance. It may be concluded that the catabolism of amino acids is increased. If we accept a frequently stated assumption that the main function of vitamin B_6 is exerted in transaminase systems, it could be concluded from the above evidence that transamination is not essential for the catabolism of amino acids nor for the production of urea.

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VITAMIN A IN HEALTH AND DISEASE*

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No attempt will be made at this time to review the vast literature on vitamin A in health and disease. Instead we will review recent experiments in my department. Our interest in vitamin A was originally aroused because of reported changes in serum vitamin A in rheumatic fever, cystic fibrosis of the pancreas and in celiac disease. The development of a micromethod for the determination of vitamin A by Bessey and Lowry and our success in adapting this method for the determination of vitamin A in urine, tissues and stool has made possible interesting studies of vitamin A in normal healthy infants and children, in patients with various diseases, and in various small animals.

In the human, the principal method of study was through the determination of fasting serum vitamin A levels and the serum levels at various intervals of time after an oral test dose of 6,000 U. S. P. units per pound body weight. Urine and various tissues were analyzed for vitamin A content. Animal experiments using dogs, rats and guinea pigs were performed to answer specific questions which arose as the work progressed.

There have been few studies of serum vitamin A levels following test doses in normal infants and children. Those reported were done for the most part on relatively few sub-

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From the Kunstadter Laboratories for Pediatric Research, the Department of Pediatric Research, and the Sarah Morris Hospital for Children of Michael Reese Hospital.

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was 32, and in the older children it was 38—a gradual increase in average fasting level with age. There was considerable overlapping between the groups, and these differences are not statistically significant. The response to the test dose however differed widely with age. The differences were sharp between the age groups just mentioned. Studies on prematurely born infants are now in progress.

The first figure shows the response to test doses in normal older children.

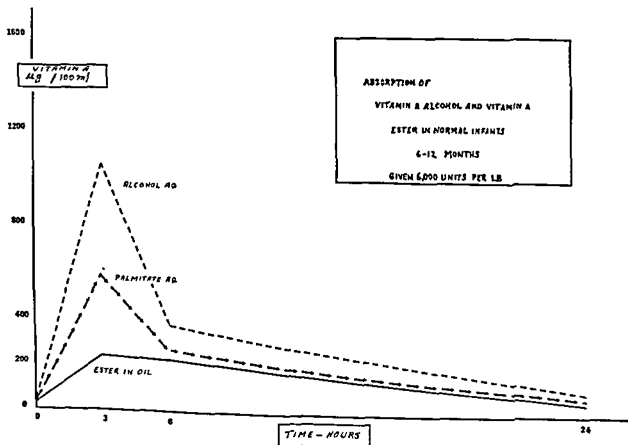


FIGURE 2. *Absorption of Aqueous Dispersions of Vitamin A Alcohol and Vitamin A Palmitate and of Vitamin A Natural Esters in Oil in Infants 6-12 Months of Age. Serum concentrations after oral dose of 6,000 U. S. P. units per pound body weight.*

jects, usually as controls for the study of a disease. It was, therefore, necessary for us to carry out a systematic and statistically significant evaluation of the normal response to test doses with the various chemical forms of vitamin A and the various media in which it may be given.

The first 3 figures show the results of our study of normal infants and children. The study revealed that the subjects (all having been normal full term infants) should be divided into three groups, first those from 1 to 6 months; second those from 6 months to 1 year, and third the older children. The average fasting level in infants under 6 months was 24 ug%; in the group 6 months to 1 year it

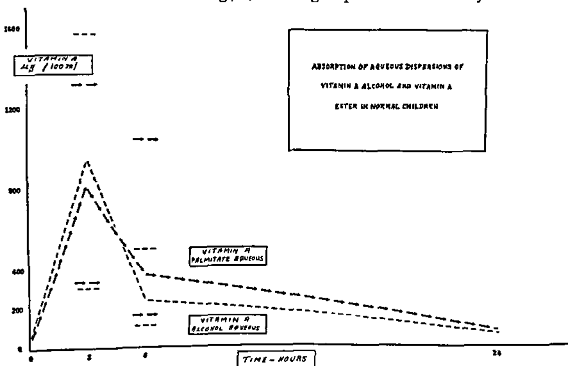


FIGURE 1. *Absorption of Aqueous Dispersions of Vitamin A Alcohol and Vitamin A Palmitate in Normal Children. Serum concentrations after oral dose of 6,000 U. S. P. units per pound body weight. Continuous lines show the mean. Others indicate the range.*

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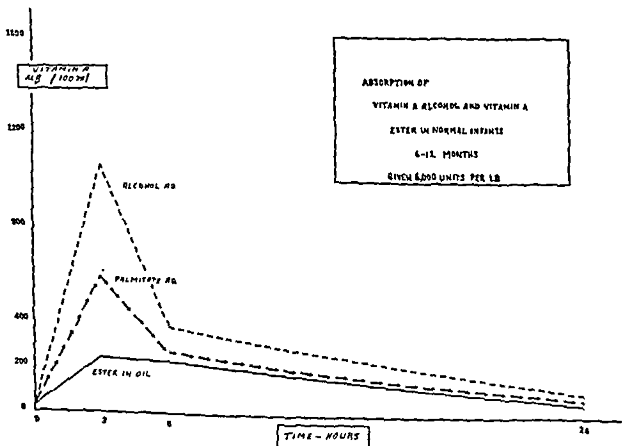


FIGURE 2. *Absorption of Aqueous Dispersions of Vitamin A Alcohol and Vitamin A Palmitate and of Vitamin A Natural Esters in Oil in Infants 6-12 Months of Age. Serum concentrations after oral dose of 6,000 U. S. P. units per pound body weight.*

You will note that there is an average rise at 3 hours to about 800 ug%; at 6 hours it is under 400 ug%; and at 24 hours it has returned to the fasting level. The highest level reached in any of the children was under 1600 ug% at 3 hours. On first examination, it would appear that *vitamin A alcohol in aqueous dispersion gave higher levels* at 3 hours and vitamin A palmitate in aqueous dispersion gave higher levels at 6 hours. Statistical analysis of the data, however, shows that this difference is not significant.

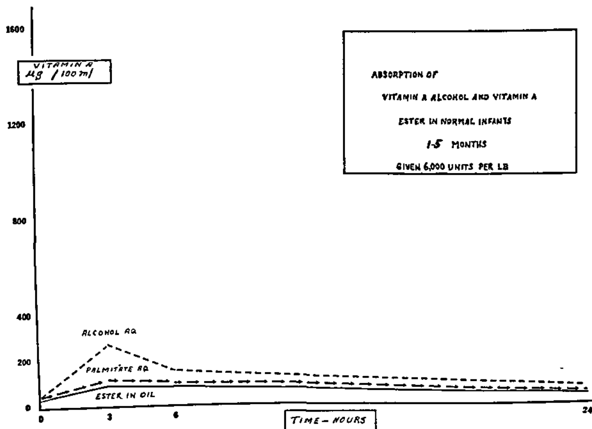


FIGURE 3. *Absorption of Aqueous Dispersions of Vitamin A Alcohol and Vitamin A Palmitate and of Vitamin A Natural Esters in Oil in Infants 1 to 5 months of Age. Serum concentrations after oral dose of 6,000 U. S. P. Units per pound body weight.*

The second figure shows the serum levels under similar conditions in infants 6 to 12 months of age. Note that at 3 and 6 hours the mean levels with the alcohol form are similar to those in the older children but that those with the palmitate in aqueous dispersion are lower. At the 3

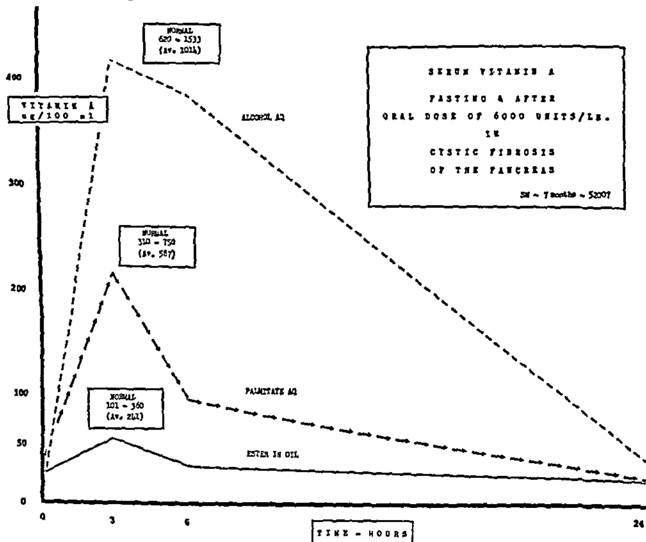


FIGURE 4. *Absorption of Aqueous Dispersions of Vitamin A Alcohol and Vitamin A Palmitate and of Vitamin A Natural Esters in Oil in 7 Month Old Infants with Cystic Fibrosis of the Pancreas. Serum concentrations after oral dose of 6,000 U. S. P. units per pound body weight. Normal Range and Average for the Age is shown in the Rectangles.*

hour interval, the average is well over 800 ug% with the alcohol and it is less than 600 ug% with palmitate. It is still lower with the natural esters in oil, i.e. about 200 ug%. The difference at 6 hours is not statistically significant although the relationship would still appear to hold. The fact that the alcohol reaches such a relatively higher level at 3 hours indicates that digestion of the esters in oil and even of the simple palmitate ester in aqueous dispersion is delayed. This difference will show up more clearly in our studies of pancreatic disease which will be discussed shortly.

The next figure shows an even more striking difference. It is clear that infants between 1 and 5 months of age show a very low rise at 3 hours. Here again, however, the rise with alcohol is significantly higher at 3 hours than the rise with palmitate or natural esters in oil. The average rise is to over 200 with the alcohol form and less than 100 with the palmitate form in aqueous dispersion and with the natural esters in oil.*

The difference in absorption of the various forms of vitamin A is more clearly apparent in infants with cystic fibrosis of the pancreas, a disease in which the pancreatic enzymes are demonstrably absent from the gastrointestinal tract. Figure 4 shows the levels in such a case following test doses. All the cases studied show similar changes. The ordinate scale of this chart is enlarged to 4 x that of the former ones. These patients show an even greater difference in absorption between alcohol and palmitate. At the 3 hour interval absorption of all forms is below the lowest

* These differences in serum levels in the infant given these various forms of vitamin A are not interpreted as indicating that one is to be preferred over the other in the normal state. There is in fact the possibility that with increased absorption the danger of poisoning when excess doses are taken may be increased and that cases of such poisoning may then be seen at an earlier age in life than at present.

normal. At the 6 hour interval, the level with the alcohol form is within normal but those with the palmitate in aqueous dispersion and the esters in oil are relatively very much lower than normal. The pancreatic enzymes, therefore, are very important for the digestion of esters, such as the palmitate, but in their absence the alcohol form is relatively well absorbed. The high levels at 6 hours with

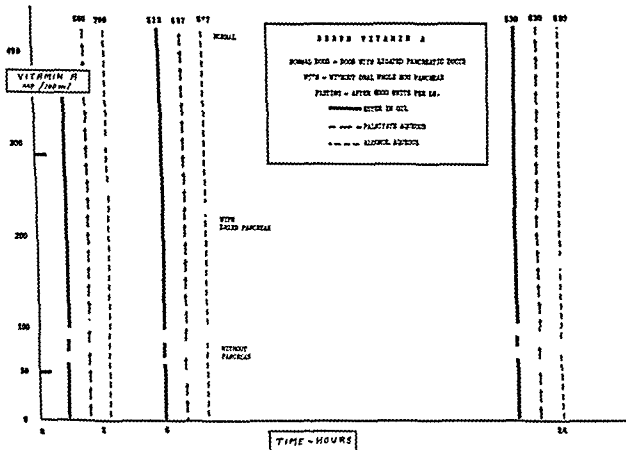


FIGURE 5. *Serum Vitamin A Levels in Dogs after Oral Doses of 6,000 U. S. P. Units per Pound Body Weight. The topmost level of the bars shows the serum levels of vitamin A in* normal dogs *at the time intervals shown. The lowest level of the bars shows the serum levels of vitamin A in* dogs with ligated pancreatic ducts NOT given hog pancreas. *The next higher level of the bars shows the levels obtained in* duct ligated dogs given whole hog pancreas orally. *The markers on the ordinate scale show the fasting vitamin A level in duct ligated dogs (about 50 ug%) and in normal dogs (almost 300 ug%).*

the alcohol form suggest some delay in its absorption. The results show that in this pancreatic disease the *chemical* form of the vitamin is more important for its absorption than the medium—that is, whether the medium is aqueous dispersion or oil.

The absorption of vitamin A in the absence of pancreatic enzymes was studied further in dogs.* Studies were done on a series of normal dogs before and 2 months after their pancreatic ducts were ligated and later while the same dogs received whole hog pancreas orally. The results are shown in Figure 5. It is clear that in the dogs with ligated ducts the alcohol form in aqueous dispersion is absorbed better than the palmitate in similar aqueous dispersion or the natural esters in oil and that there is little difference between the natural esters in oil and the palmitate in aqueous dispersion. When whole powdered hog pancreas is given to dogs with ligated pancreatic ducts, the absorption of the esters in oil and the palmitate in aqueous dispersion improves very little, but with the alcohol form there is an increase in the serum levels of vitamin A. Even with the alcohol form absorption does not approach that of the normal dog.

It appears, therefore, that in the child over one year of age a difference in absorption between vitamin A alcohol in aqueous dispersion and either vitamin A palmitate in aqueous dispersion or the natural esters in oil may be taken as suggestive evidence of the absence of pancreatic enzymes. In those under one year this interpretation is suggested if the difference in the absorption of these forms is greater than that observed in the normal. These data also show that even the relatively simple palmitate ester must be digested further and that the presence of pancreatic enzymes

* These studies in dogs were done in collaboration with Dr. Clarence Cohn and Dr. Alex Kaplan of the Department of Biochemistry of Michael Reese Hospital

is important in this digestion. They show also that vitamin A alcohol can be absorbed fairly well without these enzymes and that in the pancreatic-duct-ligated dog, administration of whole pancreas increases absorption of the alcohol form, but it does not significantly increase the absorption of the natural esters in oil or even of the simpler palmitate in aqueous dispersion. This suggests that administration of such preparations of hog pancreas to patients with this disease may contribute very little toward improving their fat absorption. Such an experimental approach offers a means of searching for better agents to be used in the treatment of the disease. That presently available pancreatic preparations do not contribute greatly to the care of these infants is the impression gained from clinical experience also.

In the course of our studies, since vitamin A is a fat soluble vitamin, we became interested in the serum vitamin A in diseases in which the serum fat content is high. We, therefore, studied serum vitamin A in cases of the nephrotic syndrome, diabetes mellitus, and hypothyroidism, in which the serum cholesterol levels, as one index, were elevated. We found the serum vitamin A levels to be normal in diabetes and hypothyroidism, but in the nephrotic syndrome the fasting levels were very high; and following test doses they characteristically rose up to 10 times higher than normal and remained high at the 24 hour period instead of returning to normal. We have found similar curves in 50 studies on 28 children with this condition.

We did *not* find these changes in acute glomerulonephritis or pyelonephritis or in most cases of chronic glomerulonephritis without the nephrotic syndrome.

We found that children with the nephrotic syndrome excrete more vitamin A in the urine than normal. The

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higher than in the normal guinea pigs, Figure 6. There was thus a change in the same direction as that observed in the nephrotic syndrome in humans. The liver vitamin A in these animals was lower than normal. In studies which are still in progress, we are finding that when phosphorus damages the parenchymal cells of the liver, similar vitamin A changes result. Furthermore when both cellular systems are impaired, the serum vitamin A changes are in similar direction but are even more marked. These changes, therefore, are not necessarily related to damage to only one cellular system in the liver but may be due to interference with the function of either or both systems; i.e. the parenchymal cells and/or the reticulo-endothelial cells.

Tissues obtained at autopsy from children who died with the nephrotic syndrome were analyzed. In view of the above animal experiments, it was reasonable to expect to find the liver vitamin A concentrations to be low. Instead we found them to be quite high—in fact from 5 to 10 times higher than normal. This could be because the liver takes it up more rapidly and/or utilizes or liberates it more slowly than is normal. Perhaps the liver is saturated with the large amount of vitamin A it contains and therefore cannot take up more.

In the course of this study we collected considerable data on the liver vitamin A concentrations in a wide variety of pathological conditions as well as in the "normal." We found that the vitamin A concentration in the central portion of the liver is higher than in either the right or the left lobes. In none of the other pathological states did we find such high liver vitamin A concentrations as in the nephrotic syndrome. The most similar changes, though not as marked, were seen in toxic or lower nephron nephrosis.

level of blood cholesterol or fatty acid did not correlate quantitatively with either fasting serum vitamin A or with the height of the vitamin A levels after test doses.

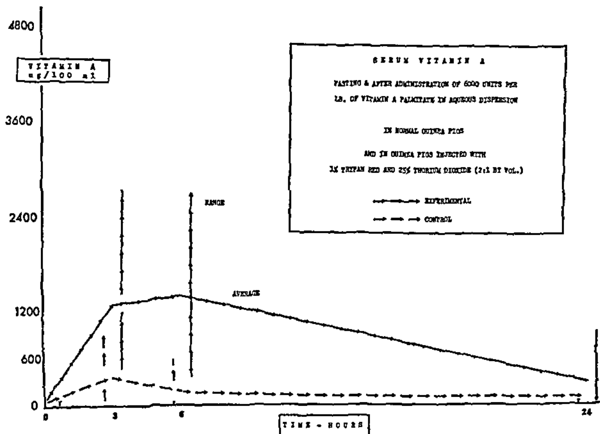


FIGURE 6.

Since vitamin A is stored mainly in the liver, the following experiments were planned to define the possible role of the liver in the abnormal utilization of vitamin A in the nephrotic syndrome. We interfered with the reticulo-endothelial cells of the liver of guinea pigs by injection of thorotrast and trypan red. After orally administered test doses of vitamin A, the serum levels in guinea pigs with impaired reticulo-endothelial systems were significantly

his long bones showed clear evidence of poisoning. One cannot, therefore, rule out poisoning because there is a normal fasting level. It is necessary in such cases to study the response to a test dose.

None of the patients with the nephrotic syndrome showed evidence of the clinical condition we know as hypervitaminosis A, either in symptomatology or on X-ray examination. This supports the theory that vitamin A although stored in the liver in the nephrotic syndrome is not being metabolized normally in other tissues or that it is in the blood stream in a form which is not readily used by the tissues.

Infections are known to lower the serum vitamin A concentration. We, therefore, thought some light on this problem could be shed by a study of the effect on serum and liver vitamin A of abscesses simulating infection. Rats were therefore given intramuscular or subcutaneous injections of turpentine or sweet almond oil. These experiments showed that in the presence of such abscesses, although the serum level does become lower, the liver vitamin A is not changed. Analysis of various kinds of tissue from the center and periphery of the abscess in the rat showed no significant change in vitamin A in any one area over the normal for that tissue.

We found that within 24 hours after removal of both kidneys, fasting serum vitamin A and the response to test doses were normal. This held true in spite of rising serum urea nitrogen. Furthermore there were no changes in the liver vitamin A in these animals.

Whether the vitamin A changes in the liver in the nephrotic syndrome are of primary or are of secondary significance remains for further studies to determine. Albanese showed that the amino acids in nephrotic serum albumi

We then considered the possibility that vitamin A is not being metabolized properly in other tissues than liver or that it is in some abnormal form in the blood stream in the nephrotic syndrome. We, therefore, turned our attention to hypervitaminosis A.

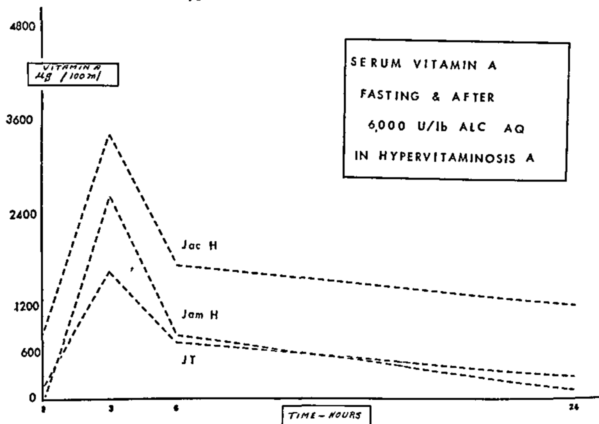


FIGURE 7.

Figure 7 shows similar studies in 3 of the cases of hypervitaminosis A in children. The curves are very similar to those seen in the nephrotic syndrome. James H., brother of Jack, had a fasting level within normal range but an abnormally high curve. It is of more than passing interest that the 2 with high fasting levels had symptoms but that though James had no symptoms, on X-ray examination,

absorption of the alcohol form somewhat but does not increase absorption of the palmitate in aqueous dispersion or of the esters in oil.

In the nephrotic syndrome, the fasting serum vitamin A is very high, and following all forms of vitamin A the levels rise much above the normal and remain high at the 24 *hour period. The evidence indicates that the vitamin A* changes in the nephrotic syndrome may be due to a defect in liver function; to an abnormal state or chemical form of vitamin A in the blood or to abnormally poor utilization of vitamin A in other tissues or a combination of these factors. Although the serum vitamin A levels in the nephrotic syndrome are similar to those observed in hypervitaminosis A, there is no evidence of vitamin A poisoning in cases of the nephrotic syndrome.

When the parenchymal cells of the liver are damaged or when the function of the reticulo-endothelial cells is impaired, serum vitamin A arises as in the nephrotic syndrome. However, liver vitamin A falls. In contrast, in the nephrotic syndrome both the serum vitamin A and the liver vitamin A rise markedly. When abscesses are produced in animals, the serum vitamin A falls but the liver vitamin A remains normal.

differ from normal, and recent evidence from England shows that the molecular weights of some portions of serum albumin in the nephrotic syndrome are not normal. On the other hand Gitlin found that nephrotic albumin and normal albumin were immunologically the same. One might speculate as to whether the origin of the possibly abnormal albumin in the nephrotic syndrome might be in a malfunctioning liver since this organ appears to be the site of synthesis of serum albumin.

In summary, we have found that serum vitamin A levels following a test dose are significantly different in the different age periods, being lowest in infants under 6 months of age, higher in those 6 to 12 months of age, and highest in the older child.

In the infant under 1 year of age, higher values are observed with vitamin A alcohol than with vitamin A palmitate even though both are in similar aqueous dispersion. Absorption of vitamin A alcohol in aqueous dispersion and vitamin A palmitate in aqueous dispersion is the same in the older child. Serum levels of the natural vitamin A in oil are lower than after either the alcohol or the palmitate forms in aqueous dispersion.

In infants with cystic fibrosis of the pancreas, there is a striking difference in the absorption of the alcohol and the palmitate forms in aqueous dispersion; the alcohol being much more rapidly absorbed. The palmitate in aqueous dispersion and the natural esters in oil are both relatively poorly absorbed in this condition.

In dogs with ligated pancreatic ducts, administration of vitamin A alcohol together with whole pancreas, while providing the highest absorption, still does not produce vitamin A levels equal to those observed in the normal dog. Giving hog pancreas orally to these duct ligated dogs improves

NEUROPATHOLOGY IN VITAMIN B_{12} DEFICIENCY*†

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The identification of vitamin B_{12} with the anti-pernicious anemia factor and the clinical observation that neurological disturbances, which may or may not be present in this disease, frequently improve or clear up following the administration of B_{12}, has led to the employment of this vitamin in the treatment of other neurological disturbances. Vitamin B_{12} has been found to be useful in the treatment of various neuropathies and neuralgias, such as trigeminal neuralgia (tic douloureux), post-herpetic neuralgia, tabetic neuralgia, the period immediately following the acute phase in poliomyelitis, phantom limb pain, diabetic neuritis and other instances of painful syndromes with obscure etiology.

One of the biochemical mechanisms of vitamin B_{12} is its role in transmethylation[1], a role that is important to the relationship of choline and methionine. Since choline is an important constituent of lecithin and sphingomyelin there is undoubtedly some relation between choline, vitamin B_{12} and the myelin sheaths of myelinated nerves, both sensory and motor. This relation may be expressed in the phospholipid or phosphoprotein metabolism in the formation and the maintenance of the myelin sheaths. Bach[1] reasoned that vitamin B_{12}, by its presence in the metabolic

* The author wishes to acknowledge the active participation of Mr Earl G Hamel and Mr J P Wack in these studies

† The author wishes to express his thanks and deep appreciation to The National Vitamin Foundation, Incorporated for the grant-in-aid which enabled this work to be carried out

supporting cells are rather difficult to differentiate from the nerve cells.

Using appropriate histochemical technics we[7] have observed that vitamin B_{12} increases the PNA content in anterior column cells in the spinal cord and in sympathetic ganglion cells in weanling rats fed a diet deficient in folic acid, para-aminobenzoic acid and B_{12}. Draper, Sime and Johnson[8] have reported demyelination and obscurity or loss of the neurokeratin (pseudo-neurokeratin) network in the peripheral nerves taken from calves fed a diet deficient in vitamin B_{12}.

In order to further elucidate on the effects of vitamin B_{12} deficiency in the nervous system, an experiment was conducted by placing weanling white rats from the St. Louis University colony on a basal diet deficient in vitamin B_{12} only. The diet used was modeled after the one used by Hogan, O'Dell and Whitley[9]. Soybean oil meal was the protein source, but there was added the "complete vitamin B mixture" in which vitamin B_{12} is not incorporated. A control group (No. I) was fed the regular laboratory chow ration. The animals placed on the basal diet were arranged in three groups. One group (No. IIA) was fed the basal diet supplemented with 3mcgm. vitamin B_{12}* subcutaneously every other day. A second group (No. IIB) was fed the basal diet supplemented with 12 mcgm. B_{12} subcutaneously every other day and a third group (No. III) was fed the basal diet only. All animals were allowed to feed *ad libitum*. Weight records were kept. Four animals, one from each group, were sacrificed at the beginning of the fourth week of the experiment and each subsequent week through the twelfth. Blood samples were taken weekly beginning the fourth week and continuing through the tenth week in order

* The vitamin B_{12} used in these experiments was furnished through the courtesy of Merck & Co, Inc and E R. Squibb and Sons.

cycle, aids in or promotes the synthesis of labile methyl groups from a carbon source. In rapidly growing young animals this synthetic mechanism may not be rapid enough to supply the demands of such growth, hence the amount of vitamin present will determine the availability of methyl groups from other biological substances giving rise to mono-carbon compounds which could in turn serve as immediate sources for the methyl groups. A similar, though less dramatic, process could be present in the adult animal when subjected to vitamin B_{12} deficiency.

The cytological localization of the activity of this vitamin is as yet not completely known. It has been demonstrated to be related in some manner to nucleoprotein, particularly and possibly only to pentose (ribose) nucleoprotein. This relation to PNA has been demonstrated in the liver and red bone marrow[2 3 4]. This relationship, however, may or may not be the same in other tissues, particularly nerve tissue Furthermore, it may or may not be the same in young and developing nerve tissues and adult nerve tissues.

Relatively little is known about the histological effect of vitamin B_{12} deficiency on nerve tissue. O'Dell, Whitley and Hogan[5] have reported the appearance of hydrocephalus in the newborn of vitamin B_{12} depleted female rats. There was a subsequent reduction of this pathology when adequate B_{12} was administered. This hydrocephalus was caused by an almost complete or complete closure of the cerebral aqueduct[6]. In the region of the brain surrounding this aqueduct, in hydrocephalic young, the cells show more desoxypentose nucleic acid than do the corresponding cells of normal newborn rats This phenomenon may be due to overgrowth of the supporting elements in this region rather than to nerve cells. At this age the

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to make red cell counts and hemoglobin and hematocrit determinations*.

The growth curves obtained were consistent with those reported by others. The animals fed the 12 mcgm. B_{12} supplemented diet grew a little more rapidly than the control group through the sixth week when the two growth curves became practically identical (Figure 1).

The spinal cord, the superior and inferior cervical sympathetic trunk ganglia, the sciatic nerves and small pieces of liver were removed from each animal and fixed in formol-saline. Some of the animals were perfused with formol-saline, before removal of the tissue. The Azur A, (Pollister[10]), and Einarson's[11] gallocyanin-chrome alum technics were used to distinguish the nucleic acid content in the tissues. Frozen and carbowax sections were also made of the above tissues and examined for lipids by the use of Sudan Black B and Scharlach R.

Observations on Nucleic Acids

Caspersson[12], Hyden[12], Gersh and Bodian[13], Bodian[12], Einarson[11], Davidson[12], Pollister[10], Pollister and Mirsky[14] and many other investigators working along similar lines support the premise that the nucleic acid content of a cell is an indication of the protein metabolism within the cell. According to Haurowitz[15], "Protein synthesis is particularly intense in those parts of the cell where ribonucleic acid is abundant". Thus, any changes in the ribo-

* As these blood determinations were not done by ourselves we cannot be too certain of their accuracy However, they do indicate a trend The figures quoted here are arithmetical averages No attempt has been made to analyze them statistically Group I rbc 6 80, hgb 14 9, hemat 59; group IIA: rbc 7.45, hgb 15 3, hemat 53, group IIB rbc 7 01, hgb 14 8, hemat. 63, group III. rbc. 6 43, hgb 13 7, hemat 48 On the basis of these figures, the red cell counts are probably within the normal ranges, whereas the hgb. and the hematocrit values for group III are lower than for the other groups

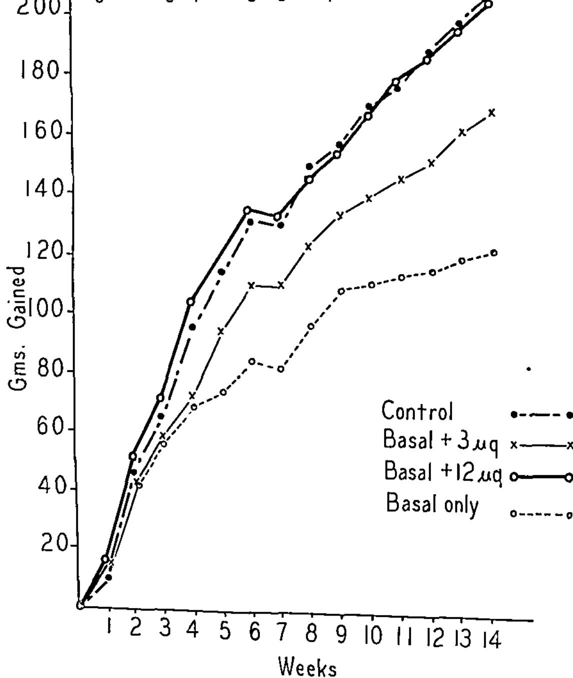

WEIGHT GRAPH-VITAMIN B12 EXPERIMENTAL RATS
Figures are group averages gained per week
220
200
180
160
140
120
100
80
60
40
20
Gms. Gained
1 2 3 4 5 6 7 8 9 10 11 12 13 14
Weeks
Control
Basal + 3 µg
Basal + 12 µg
Basal only

FIGURE 1

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The growth curves obtained were consistent with those reported by others. The animals fed the 12 mcgm. B_{12} *supplemented diet grew a little more rapidly than the control* group through the sixth week when the two growth curves became practically identical (Figure 1).

The spinal cord, the superior and inferior cervical sympathetic trunk ganglia, the sciatic nerves and small pieces of liver were removed from each animal and fixed in formol-saline. Some of the animals were perfused with formol-saline, before removal of the tissue. The Azur A, (Pollister[10]), and Einarson's[11] gallocyanin-chrome alum technics were used to distinguish the nucleic acid content in the tissues. *Frozen and carbowax sections were also made* of the above tissues and examined for lipids by the use of Sudan Black B and Scharlach R.

Observations on Nucleic Acids

Caspersson[12], *Hyden*[12], *Gersh and Bodian*[13], Bodian[12], Einarson[11], Davidson[12], Pollister[10], Pollister and Mirsky[14] and many other investigators working along similar lines support the premise that the nucleic acid content of a cell is an indication of the protein metabolism within the cell. According to Haurowitz[15], "Protein synthesis is particularly intense in those parts of the cell where ribonucleic acid is abundant". Thus, any changes in the ribo-

* As these blood determinations were not done by ourselves we cannot be too certain of their accuracy However, they do indicate a trend The figures quoted here are arithmetical averages No attempt has been made to analyze them statistically Group I rbc 6 80, hgb 14 9, hemat 59; group IIA rbc 7 45, hgb 15 3, hemat 53, group *IIB* rbc 7 01, hgb 14 8, hemat 63, group III rbc 6 43, hgb 13 7, hemat 48 On the basis of these figures, the red cell counts are probably within the normal ranges, whereas the hgb and the hematocrit values for group III are lower than for the other groups



PLATE 1 *Photomicrographs from sections of rat spinal cord stained with Azur A: (a) group I—control; (b) group IIA—basal diet supplemented with 3 mcgm. vitamin B_{12}; (c) group IIB—basal diet supplemented with 12 mcgm. vitamin B_{12}; (d) group III—basal diet only. Tenth week of experiment.*

nucleic acid ought to be a reflection of the metabolic activity in that cell. Nerve cells are rather unique. They are incapable of undergoing further division once maturity has been reached and Caspersson[12] states that the only other cells which equal or surpass nerve cells in most intensive production of protein are egg cells. Therefore, any nutritional condition that would affect protein metabolism should be manifested by observable changes within the nerve cell. The fact that not all the nucleic acid is found in the chromidial, or Nissl, substance must also be taken into consideration. Ribonucleic acid is also found in both the mitochondria and the microsomes, the latter being the most finely dispersed microscopic to submicroscopic particles in a cell.

The multipolar nerve cells in the spinal cord and the superior cervical ganglia of the supplemented and control animals show more basophilia with the two technics used than the same tissues of the non-supplemented animals, and consequently, more pentose nucleic acid (Plate 1, a, b, c, and d, and Plate 2, a, b, c, and d). The difference is quite apparent in the cells of the superior cervical ganglia, but less dramatic in the anterior column cells in the spinal cord. In most of the sympathetic trunk ganglia examined, cells in the ganglia removed from the vitamin B_{12} supplemented animals exhibited more nucleic acid than the ganglion cells removed from either the control or the non-supplemented animals.

We have been fortunate in being able to secure some human sympathetic trunk ganglia from patients who underwent two stage bilateral sympthectomy for hypertension*. The first sympathetic trunk removed served as the control.

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* We are deeply indebted to Dr Henry G Schwartz, Professor of Neurological Surgery, Washington University School of Medicine, for his cheerful cooperation and courtesy in aiding us to obtain the human material

In the interim between operations, a period varying from ten days to two weeks, the patient received subcutaneously 1000 mcgm. vitamin B_{12} daily or every other day. The total dosage varied from three to fourteen 1000 mcgm. doses of vitamin B_{12}. The ages of the patients ranged from 27 years to 51 years. Preparations of the sympathetic trunk removed after the vitamin B_{12} therapy were compared with preparations of the one removed in the first stage of the operation. The differences observed were striking, but the younger patients showed the most dramatic changes.

As soon as the sympathetic trunks were removed they were placed in fixative. The time of fixation and the time period for preparing each sympathetic trunk for sectioning was carefully controlled so that the control trunk and the experimental trunk were handled in the same way. Sections of representative trunk ganglia were stained with Einarson's gallocyanin-chrome alum and our modification of Turchini's 9-methyl-2,3,7-trihydroxy-6-fluorone[16].

The nerve cells in the control ganglia contained large clumps of chromidial granules at the periphery of the cytoplasm (Plate 3, a). The nuclei were relatively small. They were basophilic and appeared to be undergoing retrograde changes. The outlines of the cells were quite angulated and many demonstrated neuronolysis by capsule cells. In contrast, the nerve cells in the ganglia removed after the administration of vitamin B_{12}, particularly in the younger patients and in the older patients who had received the most B_{12}, showed a reduction of the neuronolysis. The basophilic staining granules were smaller and more evenly distributed throughout the cytoplasm. The outlines of the cells were more rounded and the nuclei were vesicular, a characteristic of what is usually considered to be the appearance of normal cells (Plate 3, b).

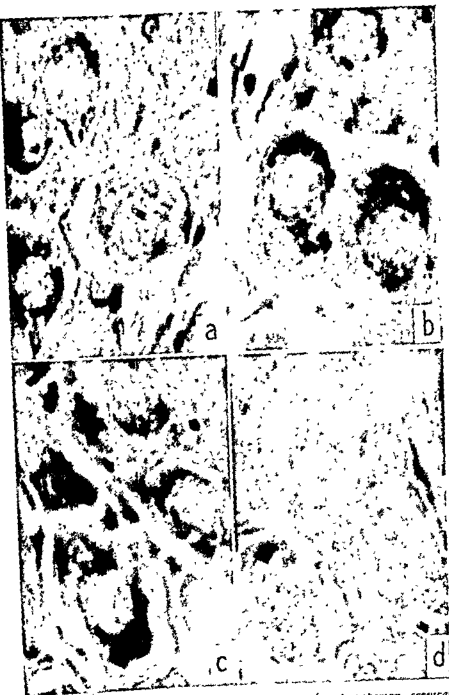


PLATE 2. *Photomicrographs from sections of rat superior cervical ganglia stained with Azur A (a) group I, (b) group IIA, (c) group IIB, (d) group III. Twelfth week of experiment*

In the interim between operations, a period varying from ten days to two weeks, the patient received subcutaneously 1000 mcgm. vitamin B_{12} daily or every other day. The total dosage varied from three to fourteen 1000 mcgm. doses of vitamin B_{12}. The ages of the patients ranged from 27 years to 51 years. Preparations of the sympathetic trunk removed after the vitamin B_{12} therapy were compared with preparations of the one removed in the first stage of the operation. The differences observed were striking, but the younger patients showed the most dramatic changes.

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PLATE 3 *Photomicrographs from human lumbar sympathetic ganglia stained with Einarson's gallocyanin-chrome alum Patient Mrs. J. L. W., Age 43 years. (a) First trunk removed 7/12/51 (b) Second trunk removed 7/24/51. Nine 1000 mcgm doses vitamin B_{12} administered in period between removal of the two sympathetic trunks.*

More neuronolysis by the oligodendroglia was observed in the spinal cord and the sympathetic trunk ganglia obtained from the vitamin B_{12} deficient animals than in the same tissues obtained from either the supplemented or the control groups. The same process was observed in the human sympathetic trunk ganglia removed before treatment with vitamin B_{12}. The encroachment seen in these preparations was quite similar to that described by Kuntz and Sulkin[17] in sympathetic trunk ganglia removed at autopsy and obtained from humans suffering various pathological conditions as well as in experimental animals whose sympathetic trunk ganglia had been stimulated for long time periods. In many instances the remnants of neurons may be seen, both in the spinal cord and in the superior cervical ganglia from the group III animals. When the total number of neurons contained in these ganglia was compared with the total numbers of the neurons in the ganglia of the control and the supplemented animals there was a definite reduction. Therefore, in addition to, or as a process accompanying the nucleic acid changes in nerve tissue, there was an increase in the destruction of cells. This presents an interesting aspect to the capsule cells or oligodendroglia, especially in the sympathetic ganglia. These cells may have more significance than has been hitherto attributed to them. They may play a significant role in relation to the metabolism of the nerve cells.

Kuntz and Sulkin observed that the capsule cells increased in number and that neuronolysis increased when the ganglion cells in the superior cervical ganglion were exhausted by prolonged preganglionic stimulation. When the capsule cells were examined with the routine stains they appeared to be arranged in a single, solid layer surrounding the ganglion cell, with the ganglion cell processes projecting through it. However, when a special stain such

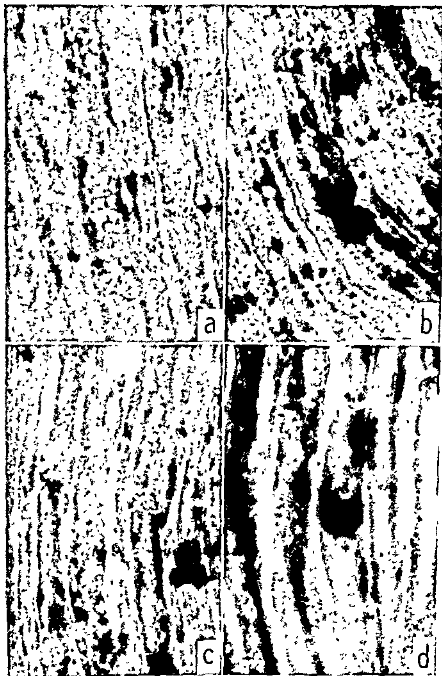


PLATE 4. *Photomicrographs from frozen sections of rat sciatic nerves stained with Sudan Black B; (a) group I, (b) group IIA; (c) group IIB; (d) group III. Tenth week of experiment.*

as ferric gallate was used, these capsule cells and their processes seemed to form a fenestrated membrane around the ganglion cell. Obviously nutritive and waste materials must traverse this membrane. Nutritive substances must be transmitted by these cells to the ganglion cell. In deficiency states, such as that produced by lack of sufficient vitamin B_{12}, these capsule cells might conceivably encroach on the cytoplasm of the nerve cell in order to procure elements necessary for their own metabolism at the expense of the nerve cell. This process may eventually lead to complete disappearance of the nerve cell. This hypothesis is interesting and not unreasonable, however, its confirmation requires further experimental evidence.

The myelinated fibers in the sciatic nerves of the non-supplemented group III animals exhibited an obscurity of the neurokeratin network. More lipid was present in the myelin of the internodal segments and at the nodal junctions. The globules of lipid were present in greater numbers and stained more intensely in the myelin in the nerves of the vitamin B_{12} deficient animals than in those of the controls (Plate 4, a, b, c, and d). This was shown in frozen sections by both the Sudan Black B and Scharlach R stains. In another technic, the nerves were fixed in Orth's fluid by perfusion and chromed for three days in 3% potassium dichromate solution. Sections were made by the usual paraffin technic and were stained by Sudan Black B in propylene glycol. The neurokeratin network was not visibly changed. By the use of the above technic the more complex lipids were fixed in the tissue, whereas the more simple lipids were removed by the lipid solvents used in the paraffin technic, *i.e.*, alcohol and xylene. This could explain the large vacuolar spaces observed in the internodal segments and at the nodes in these preparations (Plate 5, a, b, c, and d).

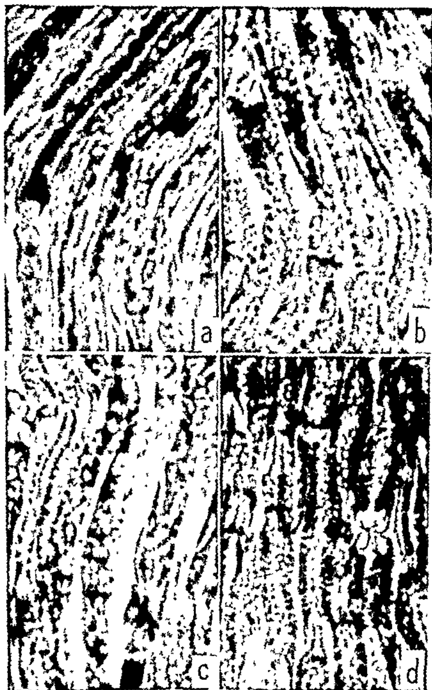


PLATE 5. *Photomicrographs from paraffin sections of rat sciatic nerves fixed in Orth's fluid, post-chromed and stained with Sudan Black B in propylene glycol: (a) group I, (b) group IIA, (c) group IIB, (d) group III. Fourteenth week of experiment*



PLATE 6. *Photomicrographs from frozen sections of rat spinal cord stained with Sudan Black B (a) group I, (b) group IIA, (c) group IIB, (d) group III. Tenth week of experiment.*

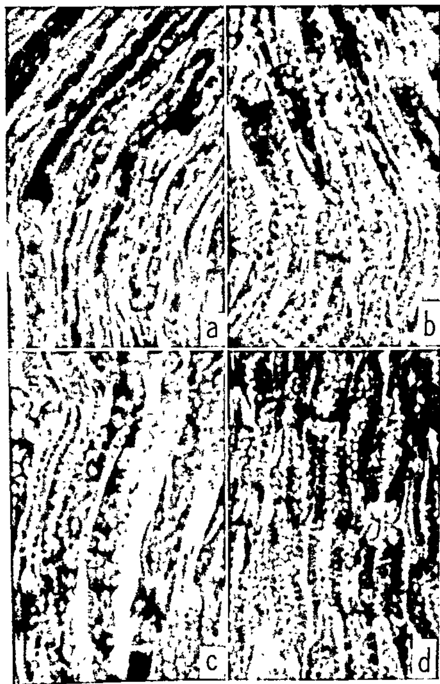


PLATE 5. *Photomicrographs from paraffin sections of rat sciatic nerves fixed in Orth's fluid, post-chromed and stained with Sudan Black B in propylene glycol (a) group I, (b) group IIA, (c) group IIB, (d) group III. Fourteenth week of experiment.*

The definite reduction in the pentose nucleic acid observed in the autonomic ganglion cells and the similar reduction, though to a lesser degree, observed in the anterior column cells in the spinal cords of the B_{12} deficient animals, as well as the increase in pentose nucleic acid observed in the treated animals, points to a relationship between vitamin B_{12} and protein metabolism. These observations on pentose nucleic acid corroborate the previous findings of other authors in other tissues (liver and bone marrow) and our own previous findings in nerve tissue. The Einarson technic has been particularly valuable because, in addition to showing the nucleic acids, it also shows the protein combined with the nucleic acid***. It appears certain, therefore, that the data obtained by the use of this technic are valid in relation to the pentose nucleic acid changes and the underlying protein moiety which is directly related to the nucleic acid.

The observations made on the lipids in the peripheral nerves are not in full agreement with those of Draper, Sime and Johnson. This may be due to species differences or differences in technic. However, we were unable to confirm a loss of the neurokeratin network. It was obscured by the presence of excess lipid. The lipid changes that occur in the myelinated nerves can probably be attributed to the effects of the vitamin B_{12} deficiency on the neurilemma cells and their counterpart, the oligodendroglia, in the central nervous system. There has been lack of agreement relative to the role of these cells in the production of myelin. The data available support the assumption that they do play an important role in the formation as well as in the maintenance of myelin. The capsule cells in the autonomic ganglia

*** Unpublished data submitted to Stain Technology for publication. Paper read before the 7th annual meeting of the Cajal Club, March 24, 1953 at Columbus, Ohio by G. Clark et al

The multipolar cells in the anterior columns of the spinal cord removed from the B_{12} deficient animals show more sudanophilic staining material surrounding the nucleus and scattered throughout the cytoplasm than do the cells from the same regions in the supplemented or control animals (Plate 6, a, b, c, and d). When these same preparations were studied with the aid of the oil immersion lens, changes in the small myelinated fibers surrounding these cells were demonstrated that were quite similar to those shown by the peripheral nerves. The myelin was definitely in a different form when compared with either the control or supplmented myelin. The characteristic ring-like appearance of the myelin sheath when viewed in cross section which may be seen in specimens from animals in group I or IIB were obscured. The outline was indistinct and fuzzy. They gave the impression of too much lipid present which was unorganized or unincorporated into the ring form normally seen.

Discussion and Conclusion

The experimental data set forth indicate that vitamin B_{12} deficiency influences both protein and lipid patterns in nerve tissue. Whether these changes are separate or concomitant, that is as lipid and protein, or as lipoprotein, only subsequent research can answer. It must be kept in mind that chemical analyses of isolated mitochondria and of isolated microsomes and presumably of chromidial granules in nerve cells, which may be only an aggregation of either of the above two when examined in fixed tissues, have demonstrated a high concentration of lipid. It may be that by the use of these histochemical technics we are witnessing the manifestation of a conjugated substance rather than two separate ones

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have been identified as oligodendroglia. These cells also probably play a very important role in the metabolism of the autonomic ganglion cells.

On the basis of the data obtained in the present study and the evidence that vitamin B_{12} plays a role in transmethylation it seems probable that its absence could cause defects in the myelin sheath pattern both centrally and peripherally. The lipid changes seen in the nerve cells, though more difficult to explain, are undoubtedly related to the same mechanism. Whether the total lipid effect is a direct one or an indirect one through the liver can be determined only by further research.

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REPORT OF ELGIN PROJECT NO. 3 WITH EMPHASIS ON LIVER DYSFUNCTION*√

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Elgin Project No. 3 has been a search for information about man's needs for nicotinic acid and tryptophan in diets which were both adequate and deficient in their riboflavin content. This report will give a summary of the organization of the dietary phases of the nicotinic acid study but we shall concentrate our attention on the changes which occurred in liver function as a consequence of a dietary deficiency which is probably not associated with nicotinic acid, but which, like kwashiorkor, is ameliorated by a diet containing liberal amounts of animal protein. Special attention will be given to changes in blood lactic and pyruvic acids and to the bromsulfalein retention test.

Experimental

General Methodology

As in our previous long term nutritional studies[1-6], the diets used were tested and stabilized in a three month preliminary period during which time the general suitability and habits of appetite of our subjects were determined. The basal diet finally chosen (Table I) provided approximately 2300 calories, 6.5 gm of protein nitrogen, 93 gm

* The author wishes to acknowledge the active participation of Drs. W. S. Rothwell, H. H. Hiatt, C. C. Harvey and R. M. Kark in this study. Dr. R. M. Wilder was chairman of the sponsoring Food & Nutrition Board Committee on Vitamin Studies at Elgin State Hospital.

√ Supported by a grant-in-aid from The National Vitamin Foundation, Incorporated.

1. U group (unsupplemented). Eight subjects received the basal diet plus 0.1 mg of riboflavin per day; this small addition of riboflavin raised the intake of this vitamin to the level used in Elgin Project No. 2.

2. R group (riboflavin supplemented). Eight subjects received the basal diet plus 2 mg of riboflavin daily.

3. NR group (*niacin and riboflavin supplemented*). Seven subjects received the basal diet plus 2 mg riboflavin and 10 mg nicotinic acid daily.

4. TR group (tryptophan and riboflavin supplemented). Eight subjects received the basal diet plus 2 mg of riboflavin and 50 mg of L-tryptophan. The tryptophan supplement was doubled to 100 mg after 10 weeks.

5. HD group (Hospital Diet). Nine subjects partook of the general hospital diet *ad libitum*, and were simultaneously subjected to the various procedures applied to the patients on the experimental diet.

Special facilities were available for the control of food intake. The diets were analyzed for nitrogen content every day and frequent estimations of the riboflavin and nicotinic acid content were also made. Approximately once every 2 months, a 72 hour urine collection was made, and aliquots were analyzed for nitrogen, creatinine, riboflavin, tryptophan, nicotinic acid, N-methyl nicotinamide, 6-pyridone of N-methyl nicotinamide and quinolinic acid. Simultaneous estimations of the glucose, and lactic and pyruvic acids in the blood after glucose ingestion were made in accordance with previously described techniques which included the

of fat, 5.8 mg nicotinic acid and 265 mg of tryptophan per day. The average riboflavin content of the basal diet was 414 mcg. The details of the constituents of the diet and the plan of feeding will be reported elsewhere. The differences between the basal diets used in this project and that used in the previous studies on thiamine and riboflavin are mainly in the protein content, which was decreased about 15 gm to a level of approximately 40 gm per day. Zein accounted for 7 of these 40 gm and gelatin 3.5 gm in order to achieve a lower tryptophan content without using the more controversial corn meal products. All the subjects on the experimental diet received a daily vitamin supplement of 30 mg ascorbic acid, 4,000 units of vitamin A, 400 units of vitamin D, 0.6 mg of thiamine, 1.0 mg pyridoxine, 3.0 mg of Ca pantothenate, 1.0 mcg B_{12}, 0.1 mg folic acid and 0.05 mg biotin. 0.5 gm of dicalcium phosphate and 0.25 gm ferrous sulfate were given three times a week. There was no supplement of alpha tocopherol but the diet did provide 40 gm of margarine per day.

Table 1
Basal Diet in Elgin Project No. 3

Protein N	6.5 gm
Fat	93 gm
Calories	2300
Riboflavin	414 mg
Niacin	5.8 mg
Tryptophan	265 mg

Forty subjects were divided into five groups as follows:

Table 2
Dietary Groups

U	Unsupplemented. Basal diet plus 0.1 mg riboflavin.
R	Basal diet plus 2.0 mg riboflavin.
NR	Basal diet plus 2.0 mg riboflavin and 10 mg niacin.
TR	Basal diet plus 2.0 mg riboflavin and 100 mg tryptophan.
HD	Hospital diet.

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estimation of the so-called Index of Carbohydrate Metabolism. The liver function tests used will be discussed later. The number of clinical, physiological and psychological tests applied were a credit to the fertile imagination of our advisors as well as to the industry of the clinicians who conducted a continuous round of physical examinations. In the clinical examinations, our local talent was checked frequently by qualified specialists who, in accordance with our established policy, are called in to check our interpretations whenever an observation of possible significance is made. We do not have the time today to give a complete description of the many tests used; most of these will be described in detail elsewhere.

Results and Interpretation

Riboflavin Deficiency

In discussing the results of this work, it should be stated at the outset that the two classical signs of pellagra, viz., diarrhea and symmetrical dermatitis, were not observed in these subjects. Definite symptoms of ariboflavinosis, as evidenced by angular stomatitis, magenta glossitis and/or scrotal dermatitis were noted in 6 of the 8 subjects in the U group who were receiving only 0.5 mg of riboflavin per day. The development and resolution of these lesions was almost identical with the course described in Elgin Project No. 2. Since the present riboflavin deficient diet differed from that in the former project in that the more recent diet was supplemented with B_{12} and unsupplemented with nicotinic acid, one may conclude that neither B_{12} nor nicotinic acid were involved in the production or amelioration of the lesions described. The U group was supplemented with riboflavin after the 39th week on the diet so that, in effect, the U group and the R group became identical.

There is much more to be said about our studies of riboflavin and nicotinic acid metabolism, but this too will have to wait for some other opportunity while we use the remainder of the allotted time for a discussion of a metabolic disorder which became evident in all the experimental groups except those on the hospital diet.

Rat Experiments

When the experimental diet plus the supplement fed the TR group was homogenized and fed to male weanling rats of the Sprague-Dawley strain, there was a marked retardation of growth which totaled 89 gm in 70 days, as compared with that of litter mates fed Purina Laboratory Chow which gained 312 gm in the same period. The livers of the animals on the experimental diet showed intense fatty infiltration. When 1 gm of lactalbumin was given as a daily supplement to the rats on the experimental diet their growth was accelerated to a normal optimal rate and the fat in the liver was almost completely mobilized. When choline citrate (10 mg/day) was added as a supplement there was a moderate diminution in liver fat but no acceleration of growth rate. Supplements of thiamine (250 mcg/day), L-lysine (100 mg/day), and ammonium citrate (0.5 gm/day) had no ameliorating effect. *Fig. I* shows results of an attempt to quantitize the effect of different amounts of lactalbumin on the growth rate of rats subsisting on the homogenized TR diet. The protein content of the human diet on a dry basis was approximately 9.0%. The addition of 40 gm of lactalbumin raised the protein content to approximately 17%. Table 3 shows the amount of fat found in the livers of the animals.

estimation of the so-called Index of Carbohydrate Metabolism. The liver function tests used will be discussed later. The number of clinical, physiological and psychological tests applied were a credit to the fertile imagination of our advisors as well as to the industry of the clinicians who conducted a continuous round of physical examinations. In the clinical examinations, our local talent was checked frequently by qualified specialists who, in accordance with our established policy, are called in to check our interpretations whenever an observation of possible significance is made. We do not have the time today to give a complete description of the many tests used; most of these will be described in detail elsewhere.

Results and Interpretation

Riboflavin Deficiency

In discussing the results of this work, it should be stated at the outset that the two classical signs of pellagra, viz., diarrhea and symmetrical dermatitis, were not observed in these subjects. Definite symptoms of ariboflavinosis, as evidenced by angular stomatitis, magenta glossitis and/or scrotal dermatitis were noted in 6 of the 8 subjects in the U group who were receiving only 0.5 mg of riboflavin per day. The development and resolution of these lesions was almost identical with the course described in Elgin Project No. 2. Since the present riboflavin deficient diet differed from that in the former project in that the more recent diet was supplemented with B_{12} and unsupplemented with nicotinic acid, one may conclude that neither B_{12} nor nicotinic acid were involved in the production or amelioration of the lesions described. The U group was supplemented with riboflavin after the 39th week on the diet so that, in effect, the U group and the R group became identical.

There is much more to be said about our studies of riboflavin and nicotinic acid metabolism, but this too will have to wait for some other opportunity while we use the remainder of the allotted time for a discussion of a metabolic disorder which became evident in all the experimental groups except those on the hospital diet.

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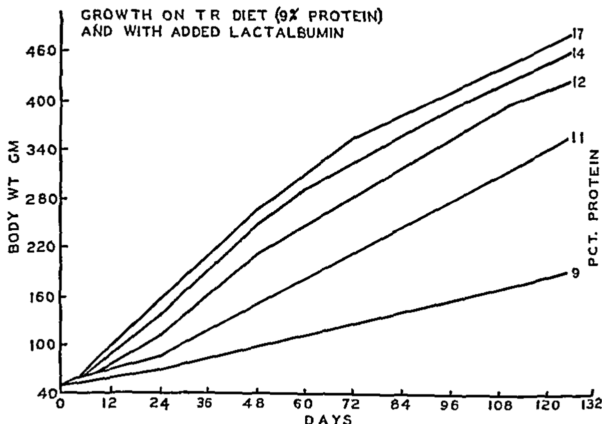


FIGURE 1.

Table 3

Liver Fat in Rats on TR Diet* for 124 days, 8 rats per group

Lactalbumin Added gm	Average Body Wt. gm	Average Liver Fat %	Range %
40	488	7.4	5.8- 9.1
24	464	6.5	5.3- 7.3
12	429	6.9	5.8- 8.3
6	361	6.9	5.5-10.2
0	194	14.3	9.3-22.4

* TR diet provides 40 gm protein per day per man on a dry basis. This is equal to 9% protein in diet. Therefore, the addition of 40 gm lactalbumin approximately doubles the protein in the homogenate fed to the rats.

Blood Lactic Acid and Pyruvic Acid

Leaving the rats and returning to the patients on the diets in question, we find that the first information that there might have been an alteration in the metabolic integrity of the subjects in the four experimental groups was noted after 7 weeks on their respective diets. At this time the blood lactic acid and pyruvic acid showed a marked increase over the pre-experimental levels. The Index of Carbohydrate Metabolism (Table 4), which is an expression of the levels of lactate and pyruvate attained after the ingestion of glucose and the application of mild exercise, increased from an average of 6.2 to 14.5 and remained high until the diet was changed.

Table 4

Index of Carbohydrate Metabolism

$$\text{CMI} = \frac{\left(L - \frac{G}{10}\right) + \left(15P - \frac{G}{10}\right)}{2}$$

where G = mg% blood glucose,
L = mg% blood lactic acid,
and P = mg% blood pyruvic acid.

Glucose (1.8 gm/kg.) is given orally. Sixty minutes later a mild exercise test, consisting of one minute of stair-climbing, is applied. Five minutes after the completion of the exercise (66 minutes after glucose ingestion) a blood sample is taken and analyzed for G, L and P.

Even more striking than this was the increase observed in the morning basal blood levels of lactic and pyruvic acids. After 7 weeks, the basal blood lactic acid had risen from an

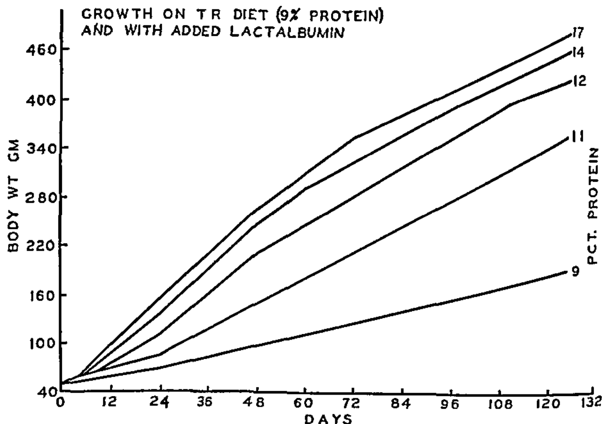


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* TR diet provides 40 gm protein per day per man on a dry basis This is equal to 9% protein in diet Therefore, the addition of 40 gm lactalbumin approximately doubles the protein in the homogenate fed to the rats.

Table 5

Index of Carbohydrate Metabolism (CMI)
of subjects in the NR group

Week:	0	46	110	190	599	711
Subject						
NR1	13.2	15.2	9.4	12.8	15.6	7.3
NR2	2.8	19.9	12.6	15.4	10.7	6.4
			17.6	14.6	15.4	8.3
			25.4	23.2	17.5	10.9
			13.6	15.4	13.3	6.8
			14.1	11.9	9.8	9.4
			17.0	14.0	18.6	12.5
			15.7	15.3		8.8

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average of 8.0 to 11.4 mg % and the pyruvic acid levels from an average of 0.84 to 1.16 mg %. These higher average basal levels generally persisted until the diet was supplemented. More significant than these averages was the frequently observed individual increases, at one time or another, in two-thirds of the subjects, to morning blood levels of more than 14.0 mg % for lactate and 1.40 mg % for pyruvate; levels which had never before been recorded in these subjects, about half of whom had been under similar observation for 2 years in the previous study on riboflavin. Such increases in basal blood levels of lactic and pyruvic acid had not been obtained even after subsistence on a diet which provided only 0.4 mg thiamine per day for over 2 years.

The first evidence which indicated that the elevated levels of lactic and pyruvic acid in the blood could be returned to normal levels by dietary means, was obtained after about 9 months on the diet when one of the subjects in the U group (U3) had an appendectomy, at which time he was taken off the experimental diet and placed on the hospital diet. Whereas his early morning basal lactic and pyruvic acids had become quite high and his index of carbohydrate metabolism had been elevated from a pre-experimental figure of 14.0 to 24.3, he reverted to his pre-experimental levels the first time he was tested after surgery. (We consider a level above 15 as being suggestive of possible pathology.)

Table 5

Index of Carbohydrate Metabolism (CMI) of subjects in the NR group

Week:	0	46	110	190	599	711
Subject						
NR1	13.2	15.2	9.4	12.8	15.6	7.3
NR2	2.8	19.9	12.6	15.4	10.7	6.4
NR3	8.7	17.0	17.6	14.6	15.4	8.3
NR4	1.4	15.1	25.4	23.2	17.5	10.9
NR5	10.6	10.4	13.6	15.4	13.3	6.8
NR6	4.9	13.7	14.1	11.9	9.8	9.4
NR7	11.6	17.2	17.0	14.0	18.6	12.5
Average	7.6	15.5	15.7	15.3		8.8

Values underlined were obtained after subjects were placed on hospital diet.

Table 5 gives the data on the CMI of the NR group as being typical of all 4 experimental groups. The underlined figures are results obtained after the subjects were placed on a normal diet containing increased amounts of animal protein. Note the marked decrease in the average result after supplementation. Table 6, for the HD group, is included to show that there were no consistent levels above 15 in these subjects who ate the hospital diet *ad lib.*

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NR3	8.7	17.0	17.6	14.6	15.4	8.3
NR4	1.4	15.1	25.4	23.2	17.5	10.9
NR5	10.6	10.4	13.6	15.4	13.3	6.8
NR6	4.9	13.7	14.1	11.9	9.8	9.4
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to quantitate the subjective reports of the examiners have been most frustrating to your reporter. Dr. Kark has given his interpretation of repetitive abdominal examinations made by himself and nine other clinicians to conclude that there was enlargement of the liver in at least 8 subjects on the experimental diet. In 3 patients the livers were firm and did not fluctuate in size. This enlargement gradually disappeared during the period of supplementation. In 5 other subjects, the palpability of the livers fluctuated while the subjects were on the experimental diet and, with supplementation, were no longer palpable. In 6 other subjects the livers were palpated but were considered clinically normal. Two patients in the HD group had records of palpable livers which have not changed to date. When the livers became palpable in 5 subjects after 6 months on the diet, the possibility that the enlargement was due to infectious hepatitis was ruled out by skin tests performed under the supervision of Dr. Capps of St. Luke's Hospital, Chicago. A diagnosis of infectious mononucleosis was also eliminated. Of some importance is the fact that 8 of the 14 subjects involved had frequent abdominal examinations during a 2 year period in the previous project without any recorded observation of liver pathology. During the second year on the experimental diet, various agents such as methionine, 330 mg/day, choline, 1.3 gm/day, and lactalbumin 30 gm/day were given as supplements for periods of 2 to 6 months, and, as was the case with the blood lactic and pyruvic acid levels, none of these had any apparent ameliorative effect on liver size. But when the subjects were placed on normal diet which provided extra large rations of meat and milk, the clinicians reported that most of the livers had markedly decreased in size within 4 months. It should be noted that the amounts of protein fed during this period was about 10 to 20 gm/day greater than that provided by the experimental diet plus 30 gm of lactalbumin.

Table 6
Index of Carbohydrate Metabolism (CMI)
of subjects in the HD group

Week:	0	110	190	228	599	711
Subject						
HD1	−0.3	4.8	−0.2		2.4	−0.8
HD2	9.6	9.0	8.4	7.8	11.4	9.2
HD3	12.0	16.1	13.0	5.9	13.2	9.6
HD4	8.2				4.4	3.8
HD5	5.9	9.7	6.8	−4.4	2.3	5.7
HD6	3.8	6.0	5.0	2.9	4.0	5.0
HD7	6.0	9.3	7.2	1.5	5.2	4.9
HD9	16.2	12.3	14.5	8.8	12.6	8.0
Average	7.7	9.6	7.8	3.8	6.9	5.7

In order to minimize the possibility that the 7 gm of zein in the diet might have had an unexpected toxic effect which raised the blood lactic and pyruvic acids, the diet of 9 of the subjects was modified, after 12 months on the experiment so that 7 gm of lactalbumin replaced the zein for a period of 2 months. No alteration in the lactate and pyruvate levels was noted as a consequence of this change in protein.

Liver Enlargement

Relatively early in the experiment, after the diet had been fed for about 5 months, Dr. Rothwell, who had been making frequent abdominal examinations, began to notice that the livers of 5 of the subjects became palpable. From that time until the present, numerous clinicians, three of whom are eminently qualified to evaluate liver dysfunction, have examined these subjects and recorded their results. Despite the fact that on any given day, there was very good correlation between the separate physical examinations by different clinicians working independently, attempts

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Table 6

Index of Carbohydrate Metabolism (CMI)
of subjects in the HD group

Week:	0	110	190	228	599	711
Subject						
HD1	-0.3	4.8	-0.2		2.4	-0.8
HD2	9.6	9.0	8.4	7.8	11.4	9.2
HD3	12.0	16.1	13.0	5.9	13.2	9.6
HD4	8.2				4.4	3.8
HD5	5.9	9.7	6.8	-4.4	2.3	5.7
HD6	3.8	6.0	5.0	2.9	4.0	5.0
HD7	6.0	9.3	7.2	1.5	5.2	4.9
HD9	16.2	12.3	14.5	8.8	12.6	8.0
Average	7.7	9.6	7.8	3.8	6.9	5.7

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Table 7

Bromsulfalein Test

Individuals in Hospital Diet (HD) Group

Week:	31	37	47	51	58	64	69	79
HD2	2.0	4.0	6.9	4.5	4.8	4.9	4.7	3.4
HD3	3.4	3.3	4.7	3.8	4.2	3.4	1.8	1.9
HD4	2.3	2.3	2.0	2.5	2.6	1.6	2.7	1.4
HD5	0.4	0.7	3.0	1.6	4.8	1.4	2.0	1.4
HD6	3.8	4.5	3.4	5.7	5.3	6.5	3.4	4.2
HD7	4.4	4.4	2.7	5.3	3.7	7.2	3.8	3.5
HD8	2.7	6.5	5.3	5.8	7.6	4.6	3.8	2.7
HD9	2.8	2.5	2.2	3.2	4.5	—	3.2	3.0
Avge.	2.7	3.5	3.8	4.0	4.7	4.2	3.2	2.7
HD1	—	—	11.8	9.8	8.7	6.4	12.1	10.0

Table 7 gives all the bromsulfalein data obtained on subjects in the HD group. One of these subjects, HD1, is a 67 year old uncooperative patient who was included in this group in order to obtain some preliminary data on old subjects. The others in this group show retentions which are essentially normal with an occasional elevated result of questionable significance. There were 14 subjects on the experimental diet whose BSP retentions were similar to those of the subjects on the hospital diet.

Table 8

Bromsulfalein Test

Subjects treated with Methionine (330 mgs/day) at 38th week and changed to Choline (1.3 gm/day) at 52 weeks

Week·	31	37	47	51	58	64	69	79
R7	7.5	7.2	7.6	6.2	5.8	*4.0	4.4	3.6
NR1	4.9	7.6	6.6	6.9	8.8	*5.2	6.2	5.0
TR8	8.2	7.2	10.8	10.0	10.0	*7.3	7.2	5.0
TR5	11.0	7.4	—	18.5	16.0	15.0	†6.7	2.0

* R7, NR1, and TR8 changed to lactalbumin supplement (30 gm/day) at 61st week.

† TR5 changed to hospital diet at 65th week.

Probably more important than the total amount of protein fed, is the fact that the amino acids in our basal experimental ration with its relatively high percentage of zein, flour, and gelatin was so poorly balanced (as a consequence of our initial objective, to decrease the tryptophan) that the addition of 30 gm of lactalbumin to this diet was much less effective than the same amount of protein added to a better balanced ration.

Bromsulfalein Test

When the possibility of liver enlargement was first encountered in our subjects, the results of the liver function tests* were reviewed. Data on serum bilirubin, gamma globulin, thymol turbidity, zinc sulfate, cephalin flocculation, and serum cholinesterase tests did not confirm the presence of faulty liver function. While looking for some other function which might be abnormal, serum cholesterol and esters, serum lipid phosphates, albumin-globulin partitions and bromsulfalein retention tests were made. Of all of these, only the bromsulfalein retention in the serum 45 minutes after injection of a dose of 5 mg/kg body weight gave evidence of any existing abnormality in the liver function of some of the subjects. Unfortunately, pre-experimental tests of bromsulfalein retention had not been performed. In an effort to compensate partially for this omission, special attention should be given to the results of the group who ate the hospital diet. It should be remembered that the food intake of most of the members of the group on the hospital diet was not controlled and our past experience has indicated that nutritional deficiencies may occur in the HD group because of poor eating habits sometimes associated with the psychotic state.

* The serum collected in Elgin was transported to Chicago where Miss D. Rix kindly compared the readings obtained against the standards used in Dr. Kark's laboratory at the University of Illinois, College of Medicine.

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HD2	2.0	4.0	6.9	4.5	4.8	4.9	4.7	3.4
HD3	3.4	3.3	4.7	3.8	4.2	3.4	1.8	1.9
HD4	2.3	2.3	2.0	2.5	2.6	1.6	2.7	1.4
HD5	0.4	0.7	3.0	1.6	4.8	1.4	2.0	1.4
HD6	3.8	4.5	3.4	5.7	5.3	6.5	3.4	4.2
HD7	4.4	4.4	2.7	5.3	3.7	7.2	3.8	3.5
HD8	2.7	6.5	5.3	5.8	7.6	4.6	3.8	2.7
HD9	2.8	2.5	2.2	3.2	4.5	—	3.2	3.0
Avge.	2.7	3.5	3.8	4.0	4.7	4.2	3.2	2.7
HD1	—	—	11.8	9.8	8.7	6.4	12.1	10.0

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Subjects treated with Methionine (330 mgs/day) at 38th week and changed to Choline (1.3 gm/day) at 52 weeks

Week:	31	37	47	51	58	64	69	79
R7	7.5	7.2	7.6	6.2	5.8	*4.0	4.4	3.6
NR1	4.9	7.6	6.6	6.9	8.8	*5.2	6.2	5.0
TR8	8.2	7.2	10.8	10.0	10.0	*7.3	7.2	5.0
TR5	11.0	7.4	—	18.5	16.0	15.0	†6.7	2.0

* R7, NR1, and TR8 changed to lactalbumin supplement (30 gm/day) at 61st week.

† TR5 changed to hospital diet at 65th week.

Probably more important than the total amount of protein fed, is the fact that the amino acids in our basal experimental ration with its relatively high percentage of zein, flour, and gelatin was so poorly balanced (as a consequence of our initial objective, to decrease the tryptophan) that the addition of 30 gm of lactalbumin to this diet was much less effective than the same amount of protein added to a better balanced ration.

Bromsulfalein Test

When the possibility of liver enlargement was first encountered in our subjects, the results of the liver function tests* were reviewed. Data on serum bilirubin, gamma globulin, thymol turbidity, zinc sulfate, cephalin flocculation, and serum cholinesterase tests did not confirm the presence of faulty liver function. While looking for some other function which might be abnormal, serum cholesterol and esters, serum lipid phosphates, albumin-globulin partitions and bromsulfalein retention tests were made. Of all of these, only the bromsulfalein retention in the serum 45 minutes after injection of a dose of 5 mg/kg body weight gave evidence of any existing abnormality in the liver function of some of the subjects. Unfortunately, pre-experimental tests of bromsulfalein retention had not been performed. In an effort to compensate partially for this omission, special attention should be given to the results of the group who ate the hospital diet. It should be remembered that the food intake of most of the members of the group on the hospital diet was not controlled and our past experience has indicated that nutritional deficiencies may occur in the HD group because of poor eating habits sometimes associated with the psychotic state.

* The serum collected in Elgin was transported to Chicago where Miss D. Rix kindly compared the readings obtained against the standards used in Dr. Kark's laboratory at the University of Illinois, College of Medicine.

These subjects, NR6 and TR4 had BSP's which remained slightly elevated, at 7.2 and 6.0 respectively, after 9 months on the hospital diet. The most consistent improvement of BSP, as with the other tests in this study, was obtained where the subjects were placed on a supervised hospital diet. Table 10 shows 4 such subjects. The data on subject U3 would not have been included in this table except for the fact that he was the first individual to be so supplemented (because of an appendectomy) and the results on *him provided a suggestion of what was to follow.*

Table 10

Bromsulfalein Test

Subjects without previous supplementation who were placed on Hospital Diet at times indicated*

Week:	31	37	47	51	58	64	69	79
U3	4.3	7.0	*4.4	3.3	3.0	2.4	2.0	1.4
U4	8.6	11.0	13.0	13.0	*11.0	7.0	7.2	4.1
NR2	15.0	12.0	13.0	10.2	*11.0	5.8	6.7	4.0
TR3	5.7	8.8	9.0	7.9	*4.8	3.6	4.4	2.6

Summary

In summary and conclusion, information obtained during Elgin Project No. 3 included the following:

1. Signs of pellagra were not noted on a diet which probably contained less niacin and tryptophan than the Goldberger prison diet[7]. The Elgin diet differed in at least one respect, it did not contain corn meal. The details of these differences and the

* U3 at 41 weeks, U4 at 56 weeks, NR2 and TR3 at 52 weeks.

The dietary supplementations attempted after liver pathology was suggested included daily additions of methionine, choline, lysine, 10 gm of lactalbumin, 30 gm of lactalbumin, and finally, natural foods which provided increased amounts of animal protein. Of these, only the 30 gm/day of lactalbumin and the supervised, meat fortified, hospital diet had any apparent ameliorative effect on the bromsulfalein retention time. The data in Table 8 indicate that additions of methionine (330 mg/day) and choline (1.3 gm/day) did not have any apparent effect on the BSP, but the addition of 30 gm lactalbumin, or a diet with increased

Table 9

Bromsulfalein Test

Subjects with elevated BSP who were supplemented with lactalbumin (30 gm/day) at 61 weeks

Week:	31	37	47	51	58	64	69	79
U1	4.0	4.6	3.8	8.8	4.2	8.4	3.0	2.4
R2	2.8	3.6	5.2	5.8	8.3	4.6	6.5	3.3
R5	3.0	3.2	2.3	4.1	9.8	2.9	2.3	2.7
R6	5.0	2.8	14.0	9.5	8.4	5.3	6.5	3.5
NR6	12.5	9.5	9.1	9.4	15.0	13.0	9.8	8.3
NR7	4.9	5.4	10.4	3.2	12.0	6.8	4.4	3.3
TR4	9.8	9.6	8.7	10.4	9.2	9.8	9.2	8.2
TR7	6.5	8.0	10.0	11.0	8.9	6.9	6.6	4.2
Avge.	6.1	5.8	7.9	7.8	9.5	7.2	6.0	4.5

protein content had ameliorative effects. Additional evidence of the possible beneficial effects of 30 gm lactalbumin per day are given in Table 9. The averages in this table are interesting even though 2 of the subjects had not been completely reversed after 18 weeks on this supplement.

These subjects, NR6 and TR4 had BSP's which remained slightly elevated, at 7.2 and 6.0 respectively, after 9 months on the hospital diet. The most consistent improvement of BSP, as with the other tests in this study, was obtained where the subjects were placed on a supervised hospital diet. Table 10 shows 4 such subjects. The data on subject U3 would not have been included in this table except for the fact that he was the first individual to be so supplemented (because of an appendectomy) and the results on him provided a suggestion of what was to follow.

Table 10

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Subjects without previous supplementation who were placed on Hospital Diet at times indicated*

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U3	4.3	7.0	*4.4	3.3	3.0	2.4	2.0	1.4
U4	8.6	11.0	13.0	13.0	*11.0	7.0	7.2	4.1
NR2	15.0	12.0	13.0	10.2	*11.0	5.8	6.7	4.0
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R5	3.0	3.2	2.3	4.1	9.8	2.9	2.3	2.7
R6	5.0	2.8	14.0	9.5	8.4	5.3	6.5	3.5
NR6	12.5	9.5	9.1	9.4	15.0	13.0	9.8	8.3
NR7	4.9	5.4	10.4	3.2	12.0	6.8	4.4	3.3
TR4	9.8	9.6	8.7	10.4	9.2	9.8	9.2	8.2
TR7	6.5	8.0	10.0	11.0	8.9	6.9	6.6	4.2
Avge.	6.1	5.8	7.9	7.8	9.5	7.2	6.0	4.5

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3. Horwitt, M. K., Sampson, Gordon, Hills, O. W., and Steinberg, D. L. Dietary management of a study of riboflavin requirements. J. Am. Dietetic Assoc. *25*:591-594, 1949.

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report of the excretion of products of nicotinic acid metabolism at different levels of dietary intake will be discussed at another time. It is important to remember that Goldberger's subjects were much more active, that they consumed more calories, and that they needed more niacin and tryptophane in consequence.

2. Since symptoms of ariboflavinosis were obtained on a diet which was supplemented with vitamin B_{12}, it can be stated that B_{12} did not noticeably affect either the development or repair of ariboflavinosis.

3. Observations on BSP retention, blood lactic and pyruvic acid and clinical diagnoses of liver enlargements have indicated that the diet used was deficient in some factor and that this deficiency was partially ameliorated by the addition of 30 gm of lactalbumin a day, but was more effectively treated by giving a complete diet.

4. The possibility that the levels of lactic acid and ketones in the blood may be an early index of liver dysfunction and/or protein insufficiency is worthy of further investigation.

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FOLIC ACID AND CITROVORUM FACTOR IN HUMAN NUTRITION*

William J. Darby
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School of Medicine
Nashville, Tennessee

Doctor Goodhart kindly invited me to discuss the subject of "Folic Acid and Citrovorum Factor in Human Nutrition." He has indicated that I might interpret broadly my assignment. Accordingly, I hope to present the viewpoint of one who has been concerned with both the basic nutritional studies relating to this factor or, more properly speaking, group of factors and with the developments and appraisal of the clinical application. I recognize that by regarding this subject from both viewpoints, I find myself at times in agreement, and at other times in disagreement, with workers in clinical medicine on the one hand and in non-medical nutrition on the other. This is inevitable, but, if my discussion can stimulate further research and sound consideration of the problems at hand, I shall feel more than rewarded.

In this discussion, no attempt will be made to assign credit or priority where there appears to be reasonable widespread accord in the findings and their interpretation. Furthermore, I shall frequently illustrate with cases and experiences of the group at Vanderbilt University results initially described by other workers. This is done only because these examples are more readily available. There is no intention of slighting the contributions made from numerous laboratories nor of trying to claim priority of observation in such instances.

* Supported by a grant-in-aid from The National Vitamin Foundation, Incorporated.

As background, may we start in the period of 1930-1935, at which time I was a medical student working as an assistant in Dr. Paul Day's Department of Biochemistry. It was my privilege there to be associated with studies on monkeys which led to the recognition of leukopenia, anemia, gingivitis, diarrhea, weight loss, and death as a syndrome which results from feeding monkeys a diet deficient in some nutrient[1]. The grosser characteristics of this nutrient were studied and evidence produced that the nutrient was not inorganic in nature[2,4] and that it differed from any of the known vitamins[2,3,4] and also from the anti-pernicious anemia factor present in purified liver extract[2,4]. Suffice it to recall that we proposed[3] "the designation vitamin M for the hitherto undifferentiated factor which prevents this nutritional cytopenia in the monkey." The similarity of the hematologic and clinical picture of these animals to a number of diseases in man, usually associated with malnutrition, was impressive and led me to return to this problem later when the opportunity was right for clinical studies with well-defined preparations.

At approximately the same time or shortly thereafter, numerous workers recognized in other species a need for a substance which eventually proved to have this same biological activity. A great mass of evidence[5,6] has accumulated indicating that the chick, monkey, fox, certain micro-organisms, and so on require this vitamin which is now frequently termed "folic acid." It is important to recall this background because too often folic acid has been regarded as a therapeutic chemical and its well-established role as an essential nutrient for several species entirely overlooked. Furthermore, in the minds of some clinicians, nutrition workers have been accused of confusing folic acid with the factor present in active liver preparations effective in the treatment of pernicious anemia. Perhaps some too-

enthusiastic vitaminologists were guilty of this immediately after the discovery of the effectiveness of these compounds in man, but the record clearly stands that these two factors were decisively differentiated by nutrition workers very early in the study of the vitamin[2,4,7].

Historically, again, by a combination of assays on monkeys and microbiological studies, the group at Arkansas pointed to the existence of at least two factors in the vitamin M group and implied that this difference might be due to the type of binding within the natural products[8]. It is now apparent[5,6,9] that in natural products there may be at least three compounds—pteroylglutamic acid, pteroyltriglutamate, and pteroylheptaglutamate—which differ merely in molecular size. Furthermore, it is apparent that certain rather specific enzymes are concerned with the liberation of free pteroylglutamic acid from the conjugated forms. These enzymes have been termed "conjugases." The conversion of the conjugated folic acids to free folic acid has been established in man. Finally, there are substances in natural products which inhibit the action of these conjugases and which are termed "conjugase inhibitors."

It was considered initially that free pteroylglutamic acid was the active form of the vitamin in man, and there was a period during which it was widely hypothesized that some defect in the conjugase enzymes existed in pernicious anemia. This seemed an explanation for the development of a so-called "conditioned deficiency" in man because of the unavailability of the bound vitamin. Such a hypothesis is not in keeping with the observation of hematologic relapses in some patients with pernicious anemia during treatment with PGA. Nevertheless, one observation, as yet uncontradicted, emerged during this period and it deserves more attention and elaboration. This is the

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enthusiastic vitaminologists were guilty of this immediately after the discovery of the effectiveness of these compounds in man, but the record clearly stands that these two factors were decisively differentiated by nutrition workers very early in the study of the vitamin[2,4,7].

Historically, again, by a combination of assays on monkeys and microbiological studies, the group at Arkansas pointed to the existence of at least two factors in the vitamin M group and implied that this difference might be due to the type of binding within the natural products[8]. It is now apparent[5,6,8] that in natural products there may be at least three compounds—pteroylglutamic acid, pteroyltriglutamate, and pteroylheptaglutamate—which differ merely in molecular size. Furthermore, it is apparent that certain rather specific enzymes are concerned with the liberation of free pteroylglutamic acid from the conjugated forms. These enzymes have been termed "conjugases." The conversion of the conjugated folic acids to free folic acid has been established in man. Finally, there are substances in natural products which inhibit the action of these conjugases and which are termed "conjugase inhibitors."

It was considered initially that free pteroylglutamic acid was the active form of the vitamin in man, and there was a period during which it was widely hypothesized that some defect in the conjugase enzymes existed in pernicious anemia. This seemed an explanation for the development of a so-called "conditioned deficiency" in man because of the unavailability of the bound vitamin. Such a hypothesis is not in keeping with the observation of hematologic relapses in some patients with pernicious anemia during treatment with PGA. Nevertheless, one observation, as yet uncontradicted, emerged during this period and it deserves more attention and elaboration. This is the

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Neurologic impairment (peripheral neuritis and combined system disease) is not infrequent in pernicious anemia but is rare in the other nutritional macrocytic anemias. Pernicious anemia is further distinguished within this group by the absence or quantitative reduction of the intrinsic factor in the gastric secretions and by the rarity of the association of the condition with the consumption of grossly deficient diets. By contrast, the other anemias of this group are frequently (but not invariably) encountered in persons consuming diets low in protein and foods of animal origin. This dietary relationship is somewhat puzzling. I have seen some persons with sprue who have had quite satisfactory intakes of foods including milk and eggs. On the other hand, I have seen diets widely consumed in regions of endemic pellagra which were very nearly devoid of protein of animal origin, of vegetables, and of fruits, yet, so far as we could find, totally unassociated with the occurrence of macrocytic anemias within the population.

Turning to pernicious anemia, this disease may be regarded as a deficiency of vitamin B_{12} conditioned by the lack of intrinsic factor which is necessary for the absorption of the vitamin. Furthermore, judging from the therapeutic response to vitamin B_{12}, this deficiency accounts for both the anemia and the neurologic defects. The pteroyl-

finding by Bethell[10] that conjugase inhibitor administered simultaneously with conjugated pteroylglutamates reduces the availability of folic acid to normal persons as well as to individuals with pernicious anemia.

It seems reasonable to assume that the conjugated pteroylglutamates must be converted to the free form by man. Hence, data bearing upon the distribution and biological potency of the conjugates, conjugases, and conjugase inhibitors must be awaited before we can obtain a clear picture of folic acid activity present in foods and the conditions which alter its availability.

In 1948, Sauberlich and Baumann[11] reported the existence of a growth factor necessary for leuconostoc citrovorum and the factor was identified as a metabolite of folic acid. Its occurrence in increased quantities in the urine of animals and man after ingestion of pteroylglutamic acid was reported[12], and it has been postulated by some that this may be the active form of folic acid, since citrovorum factor has a much greater microbiological activity for certain organisms than does folic acid. Synthetic preparations[13, 14] have the activities and properties of the substance isolated from natural sources. The greater activity of the natural material[15] appears to be due to the optical isomerism which exists, for the resolved *l*-L-leucovorin has twice the activity of the unresolved and equals the activity of the pure compound isolated from horse liver[16].

Citrovorum factor is active hemopoietically in man[17] in cases of pernicious anemia, megaloblastic anemia in infancy, sprue, and nutritional macrocytic anemia, but definitive quantitative studies of the comparative activity of this metabolite and of pteroylglutamic acid remain to be reported.

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Six of these twelve patients had exhibited at some time in their past history, prior to withdrawal of liver therapy, clinical evidence of combined system disease. No single patient developed even mild combined system disease during this relapse regime. In other words, hematologic relapse preceded in all instances the occurrence of combined system disease. Nutritionally, this may be interpreted as indicating that it requires more severe depletion of vitamin B_{12} to bring about the neurologic damage. Looking at it another way, the most essential function, maintenance of neurologic integrity, has prior call on the vitamin B_{12} over the hemopoietic system.

Comparing our series of spontaneous relapse with reports of neurologic relapse in patients treated with PGA alone there is some suggestion that neurologic disease is more frequent and more acute among those treated with PGA than in persons allowed to relapse without therapy of any kind. The difficulty of making proper comparisons is obvious, however. Folic acid in pernicious anemia acts by stimulating hemopoiesis and, hence, masks the normal time of appearance of relapse. Therefore, the usual time relationships between hematologic and neurologic relapse are altered. It is possible that the stimulated hemopoiesis uses up some of the meager stores of vitamin B_{12} present in the body, speeds up the depletion of vitamin B_{12} and,

glutamates, including citrovorum factor, will produce hemopoietic response in patients with pernicious anemia. These substances, however, do not protect against the development of the neurologic lesions. The initial observations that patients with pernicious anemia who were treated with folic acid alone frequently developed neurologic disease were interpreted by some as indicating a toxic manifestation of folic acid. Those familiar with the history of folic acid as a vitamin were reluctant to accept such an interpretation. Indeed, it has been clearly demonstrated[18] that folic acid does not possess any toxic effects when administered over considerable periods of time to healthy individuals or individuals with mild hypochromic anemia. In my own experience, the vitamin has exhibited no ill effects when administered to some patients with anemia other than pernicious anemia for periods of five years or more.

What, then, is the explanation of this sudden occurrence of neurologic damage in patients with pernicious anemia treated with folic acid?

Perhaps bearing on this question are some studies which we[19] have had under way since 1945. These observations were made on a small group of carefully and repeatedly observed patients who had pernicious anemia which had been under good control on adequate therapy with liver extract. In 1945, 12 of these patients without evidence at that time of neurologic disease were selected and all therapy deliberately withheld. Frequent hematologic and clinical studies were made at intervals of from one to four weeks. The length of time required for the appearance of a defined hematologic relapse was determined. A hematologic relapse was defined as the occurrence in a given patient of two or more consecutive erythrocyte counts which were more than two standard deviations below the

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One cannot yet account for the hemopoietic activity of folic acid in vitamin B_{12}-deficient patients with pernicious anemia. Is folic acid the factor necessary for the maturation of the megaloblast, as has been suggested by some? Is it possible that neither folic acid nor vitamin B_{12} are the factors necessary for this maturation but, instead, that some methylated metabolite is the factor and that the apparent overlapping of activity has to do with the supply of methyl groups? That is, can we attribute this effect of folic acid to its influence on methylneogenesis, if I may be permitted to use the word.

Another major influence of folic acid in man has to do with improvement of impaired gastrointestinal absorption in patients with sprue, nutritional macrocytic anemia, the megaloblastic anemia of pregnancy and in infancy. Our early observations on these improved functions[23], the studies[24] of Suarez and Spies and others, and the report by Fox[25] that patients with sprue maintained on dietary liver extract regimes were improved insofar as the gastrointestinal symptoms were concerned when transferred to folic acid, indicate that this function deserves more intensive study. In addition, there are some reports of improvement in chronic non-sprue diarrhea after treatment with PGA. The failure of some investigators to observe improved absorption from the administration of folic acid in instances of gastrectomy or of pancreatectomy in nowise applies to these observations on sprue and related conditions. Furthermore, the failure[27] of certain resistant cases of sprue to respond to folic acid after failing to respond to all other types of therapy does not detract from

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This brings us to a question as to the mechanism by which folic acid promotes hemopoiesis in pernicious anemia —a subject which has received little attention since the lag in interest in the conjugase explanation offered some years back. It is striking that, not only in the same disease but indeed in the same patient[20], one may produce remissions by liver extract, folic acid, or vitamin B_{12}. Dr. Sam Clark, Jr., working in our laboratory, has just reported[21] on studies designed to investigate the obvious hypothesis that there exists in pernicious anemia a defect in the absorption of folic acid. Because of the complication of intestinal synthesis, balance studies are not applicable for the study of absorption of this nutrient. Hence, we resorted to use of an oral tolerance test. By studying a series of dose levels it was found that 1 milligram of folic acid (pteroylglutamic acid) administered orally produced a measurable rise in the serum free folic acid in most normal individuals. This was the minimum dose which would with certainty produce a measurable rise in serum level in most subjects. Hence, this dose should provide a sensitive indicator of any defect in absorption which might appear in a disease. It was found that the maximum height attained in subjects with pernicious anemia was similar to the maxima attained in healthy persons. This is interpreted as evidence against the existence of a defect in the absorption of folic acid in pernicious anemia.

Perhaps the observation that a relatively small dose of orally administered folic acid has been remarkably efficient in treating a few cases of postgastrectomy anemia[22] may also indicate that a gastric factor is not required for absorp-

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hence, does bring on a more acute neurologic manifestation of vitamin B_{12} deficiency. One cannot decide these points at present.

This brings us to a question as to the mechanism by which folic acid promotes hemopoiesis in pernicious anemia —a subject which has received little attention since the lag in interest in the conjugase explanation offered some years back. It is striking that, not only in the same disease but indeed in the same patient[20], one may produce remissions by liver extract, folic acid, or vitamin B_{12}. Dr. Sam Clark, Jr., working in our laboratory, has just reported[21] on studies designed to investigate the obvious hypothesis that there exists in pernicious anemia a defect in the absorption of folic acid. Because of the complication of intestinal synthesis, balance studies are not applicable for the study of absorption of this nutrient. Hence, we resorted to use of an oral tolerance test. By studying a series of dose levels it was found that 1 milligram of folic acid (pteroylglutamic acid) administered orally produced a measurable rise in the serum free folic acid in most normal individuals. This was the minimum dose which would with certainty produce a measurable rise in serum level in most subjects. Hence, this dose should provide a sensitive indicator of any defect in absorption which might appear in a disease. It was found that the maximum height attained in subjects with pernicious anemia was similar to the maxima attained in healthy persons. This is interpreted as evidence against the existence of a defect in the absorption of folic acid in pernicious anemia.

Perhaps the observation that a relatively small dose of orally administered folic acid has been remarkably efficient in treating a few cases of postgastrectomy anemia[22] may also indicate that a gastric factor is not required for absorp-

from certain cases which we have observed in which megaloblastic anemia was seen to occur in infants who had high normal levels of ascorbic acid in their plasma.

These observations on vitamin C, citrovorum factor, and folic acid do seem *logically to resolve a long standing* controversy dealing with the existence of anemia in scurvy. Many workers who have seen cases of adult scurvy of the chronic "bachelor type" have observed associated anemia, as reported by Vilter and his co-workers[33]. Vilter noted that this anemia was gradually alleviated by treatment with vitamin C. The likely explanation seems to be that the development of this inconstant anemia of chronic scurvy is dependent upon a borderline supply of folic acid in the body. Indeed, Gebuzda and co-workers[34] have demonstrated that in two patients with adult scurvy the urinary excretion of citrovorum factor following the administration of PGA approached that of the healthy subject only after treatment of the scurvy with vitamin C. They concluded that one role of ascorbic acid in man is to provide for this conversion of PGA to citrovorum factor.

A related metabolic interrelationship between vitamin C and folic acid is that which concerns tyrosine metabolism. In ascorbic acid deficiency there results a tyrosyluria when excessive tyrosine is fed. This can be eradicated by giving vitamin C. We demonstrated[35] some years ago that this tyrosyluria produced in scorbutic guinea pigs could be eradicated by injecting rather large doses of folic acid. Observations on this phenomenon have stimulated much speculation. Woodruff has recently found that citrovorum factor acts similarly to PGA in the scorbutic guinea pig. From our laboratories as well as from others has come confirmation of the effect of vitamin C on tyrosyluria in infantile scurvy. Woodruff, however, found that with the doses of PGA

the positive findings, inasmuch as it has long been recognized that irreversible phases of gastrointestinal dysfunction occur in sprue. Finally, it is often impossible to differentiate certain cases diagnosed as "irreversible sprue" from instances of Whipple's lipodystrophy or other abnormalities of the mesenteric lymphatics, in the absence of either autopsy or surgical exploration.

The two conditions usually termed "pernicious anemia of pregnancy"[28] and "megaloblastic anemia in infancy"[29] seem to respond more specifically to folic acid than to vitamin B_{12}. Megaloblastic anemia of infancy responds[29, 30] at times to vitamin B_{12} but apparently invariably to folic acid. Cases refractory to vitamin B_{12} have been reported.

Most reported cases of pernicious anemia of pregnancy are refractory to vitamin B_{12} but do respond well to folic acid.

It may be that some lead to explain these variable responses in these two conditions will result from studies on the interrelationships between vitamin C, folic acid, vitamin B_{12}, and the citrovorum factor. Nicholl and Welch[31] observed that the conversion of folic acid to citrovorum factor was enhanced by ascorbic acid. May and his co-workers[32] found that monkeys fed on a milk diet without vitamin C developed scorbutic symptoms and megaloblastic anemia. The anemia could be alleviated by folic acid, or less dramatically by vitamin C. On this diet, megaloblastic anemia in the monkey did not develop when vitamin C was present in sufficient quantity. It was hypothesized, therefore, that ascorbic acid is essential for the conversion of folic acid to citrovorum factor and that the latter is the active principle in the prevention of megaloblastic anemia. While this explanation may be valid in some instances, it is not invariably so, as may be reasoned

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which he used the tyrosyluria in infantile scurvy could not be eradicated[36]. Others[37], using larger doses of the agent, have shown the PGA effect in both scorbutic infants and, inconstantly, in premature infants. I am not aware of reports on the effectiveness of citrovorum factor in this phenomenon in infants. Neither folic acid nor citrovorum factor influence the course of clinical or experimental scurvy nor the blood or tissue levels of vitamin C in guinea pigs[38]. The significance of these several findings is unclear.

From the evidence outlined here, there is no reason to doubt that folic acid and citrovorum factor are normal metabolites or metabolic substances in man. Whether normal man is dependent upon a *dietary supply* of this vitamin remains to be determined. Experimental production of a dietary deficiency of folic acid in man has not been accomplished.

Dietary studies in man of folic acid consumption do not allow one to make a reasonable estimate of usual intakes or of the intake under naturally occurring conditions of deprivation. A major need at present is a critical systematic differential study of folic acid activity of foods—a study which will define the quantity of free folic acid, of conjugates, of thymidine and other non-folic stimulants[39], as well as give information on the presence of conjugase inhibitors.

When this information becomes available it can be interpreted only in relationship to more quantitatively defined knowledge of minimal effective levels of each member of this group of pteroylglutamates. These estimates will, in turn, involve consideration of those interrelationships which I have mentioned—supply of ascorbic acid, of vitamin B_{12}, of methyl donors—and probably of other dietary

and metabolic factors which I have not discussed. For example, glycine, histidine, p-aminobenzoic acid, adrenal activity, etc. Finally, the role and importance of intestinal synthesis of the pteroylglutamates in man remain to be defined.

It can be concluded, I believe, that we are now entering the quantitative phase of study of folic acid and its relatives as these are concerned in human nutrition. The complexity of the problem should not deter us from insisting upon clear and definitive experimental evidence to serve as a basis for our conclusions and opinions.

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THE EFFECT OF DIETARY SUPPLEMENTATION AND THE ADMINISTRATION OF VITAMIN B_{12} AND AUREOMYCIN ON THE GROWTH OF SCHOOL CHILDREN*

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The vast amount of experimental work on the effects of diet on the growth of laboratory and domestic animals together with the large number of sound clinical studies leave no doubt as to the basic importance of nutrition as a major factor in the growth and development of children. Nevertheless, due to the difficulties of controlling the multiple environmental factors in human growth studies, the length of time such investigations require, the expense involved and the difficulty of evaluating genetic influences, many important questions regarding the role of nutrition in human growth and development are still unanswered.

One of the most intriguing of the unsolved problems as well as far reaching in its consequences is the role in human growth of vitamin B_{12} and other substances with animal protein factor activity. Animals fed on vegetable protein rations almost invariably grow better when one of these

* Miss Lucila Sogandares, nutritionist assigned by UNICEF, organized the study in El Salvador and carried out the diet surveys. The physical examinations were done by Dr. Adela Cabezas and Dr. Tomas Pineda In Guatemala the dietary surveys were organized by the FAO nutritionist, Miss Emma Reh, assisted by Miss Marina Flores who also supervised the supplementary snacks. The physical examinations in Guatemala were done largely by Dr. J. Antonio Munoz The project was supported by funds from the National Vitamin Foundation, Merck and Co. and Lederle Laboratories Inc. The authors are greatly indebted to Miss Gertrude Cox, Director of the Institute of Statistics of the University of North Carolina for guidance in the statistical treatment of the data and to Lilia Arroyave and Adriana Rodriguez for their work as statistical clerks. INCAP Scientific Contribution I-29.

of the administration of these substances was also included in the investigation.

Studies in El Salvador

Status of the Children

The children of both urban and rural El Salvador are approximately two years behind those of the United States in height and weight. Their bone ages, as estimated provisionally by the late Dr. Phillip Jeans in his last piece of scientific work before his death, show a similar retardation when compared to the standards of Watson and Lowry[12]. There is no way of knowing at present, whether racial factors are partly responsible for this difference although they are not believed to be the principal cause. Clinical examinations of these children reveal few of the signs usually associated with malnutrition except for the prevalence of mild degrees of xerosis and follicular hyperkeratosis. No suspected cases of rickets or scurvy were encountered. The most striking clinical observation was the discrepancy between apparent age. Approximately 28% of the rural children had mildly enlarged thyroid glands[13]. The rural groups averaged 83% blood type O and the urban groups 73%.

Hematological studies failed to reveal any important deviations from normal values. The serum total protein, riboflavin, vitamin C, vitamin E and alkaline phosphatase levels in these children have been previously reported to be within normal limits[14]. The serum levels of vitamin A and carotene were found to be relatively low, averaging 21.7 and 68 micrograms per cent respectively. Upon first examination 79 per cent of the rural children were found to have Ascaris lumbricoides, 13 per cent Necator americanus and 9 per cent Trichiurus trichiura. The incidence of these parasites was slightly lower in the urban children.

substances is added. The essential amino acids can, of course, be supplied by vegetable proteins in suitable combinations, but do children also need animal protein or one of the substances with so called animal protein factor activity for satisfactory growth?

Since large areas of the world are not at present able to furnish animal protein for human nutrition in quantities judged desirable, this question has great international, social, economic and political importance. If it can be demonstrated that the animal protein factor is *not* needed for good human growth *or* that it can be practically and cheaply supplied in a synthetic form, much benefit should result, especially to the extensive underdeveloped areas of the world in which animal protein production is low and cannot be readily increased.

Surprisingly, direct comparisons of the effectiveness of animal and vegetable protein feedings are very few in number[1-3]. Although these few comparisons fail to show an advantage of animal over vegetable protein, they are of such short duration that they do not justify recommending primarily vegetable diets for permanent use in underdeveloped areas. The published studies[4-8] which suggest a possible stimulatory effect of vitamin B_{12} on child growth are not conclusive. The same must be said of the negative reports[9-11] at least in regard to the application of their findings to all age groups.

The present study was designed to compare the effects of supplementary animal and vegetable protein on the health, growth and development of children living on a diet low in animal protein, although only the height and weight results are discussed in the present paper. Since vitamin B_{12} and the antibiotic Aureomycin act as animal protein factors in animals fed a vegetable protein ration, the effect

of the administration of these substances was also included in the investigation.

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The children were treated for their intestinal parasites at the beginning of each year. All of the children were living at an altitude of approximately 2,000 feet in a semi-tropical climate.

Experimental Design

Fifty children in each of two urban schools in the capital city, San Salvador were selected. The children in one of these schools (Colombia) were given a daily lunch containing milk and other sources of animal protein. Those in the other (Roosevelt) received no supplement. These schools were located only a few blocks apart in a poor district of the city.

Thirty-one children in one rural school (Comecayo) received the lunch containing animal protein and 50 children in another (Portezuelo), a quarter of a mile away, received a lunch free of animal protein and based on soya milk (made available by the Soya Food Research Council of the United States). Twenty-four of the children in Portezuelo received in addition 20 micrograms of vitamin B_{12} daily. A third school, Matazano less than a mile away with 22 children, served as a control. The total number of children in each group decreased each year as some families moved to new localities, but new children were also added to some of these groups in subsequent years.

The average diets before and after supplementations were determined during the first year by 7 day dietary surveys on a small random sub-sample in the urban groups and for approximately half of the rural children. An improvement on the animal protein content of the diets of children receiving the lunch containing animal protein from 15 to 32 grams in the urban school (Colombia) and 8 to 23 grams in the rural one (Portezuelo) was effected. Children of the school receiving vegetable protein (Come-

cayo) averaged 7 grams of animal protein in the initial diets. Children in the urban control school (Roosevelt) averaged 14 grams, and the children in the rural control school (Matazano) 8 grams. The initial diets were also relatively deficient in vitamin A activity (1000-2500 I. U.), riboflavin (.5-.8 mgs.) and calcium (450-650 mgs.). The supplementary feeding improved the situation in regard to riboflavin and calcium but left the diets short of the desired intake of vitamin A.

Results

When the children in the two urban schools, Roosevelt and Colombia, were compared, no effect of the lunch on the rate of gain in height and weight could be detected during any of the three years, Table I. Except for the group in Portezuelo receiving both the vegetable protein lunch and vitamin B_{12}, the children in the rural schools did not gain as rapidly in height as those in the urban groups.

Table I

Adjusted Gain in Height and Weight of School Children in El Salvador

School	Treatment	Starting Date	No. Children	Interval in Months	Average Monthly Gain* Height Cms	Average Monthly Gain* Weight Kgs
		URBAN				
Colombia	Animal Protein Lunch	II-1950	23	31	.48	.27
Roosevelt	Control	III-1950	30	30	.49	.26
		RURAL				
Matazano	Control	VII-1950	12	25	.41	.21
Comecayo	Animal Protein Lunch	V-1950	22	28	.40	.20
Portezuelo	Veg. Protein Lunch	V-1950	14	28	.40	.20
Portezuelo	Veg Protein Lunch + B_{12}	V-1950	17	28	.48	.20

Approx L. S D.$_{.05}$ for HT = .11 Approx. L. S. D.$_{.05}$ for WT = .09

* The data in this and subsequent tables are adjusted by multiple regression methods for differences in initial age, height and weight.

To the intense disappointment of all concerned with the project, the relatively good animal and vegetable protein containing lunches supplied at considerable effort and expense to the children in the two rural schools Comecayo and Portezuelo produced no measurable improvement in the rates of gain in height and weight over those of the rural control group. The addition of vitamin B_{12} appeared to have no effect on weight gain, but appeared to produce a moderate effect on gain in height over the twenty-eight month period. The over-all rate of gain in this group was similar to that found for children in the two urban schools.

Comment

After calculation of the diets, a process greatly retarded by the necessity of analyzing many hitherto unstudied local foods and the preparation of a food composition table, it became obvious that the amount of vitamin A received by the children was relatively low even after supplementation. It is possible that this interfered with the growth response of the children to the various supplements, but we have no direct evidence on this point. It should be noted that since the lunch provided substituted for the main meal of the day at home, it was difficult to bring about much quantitative improvement in the diets by these means. We also do not know at this time whether the children responded favorably in ways not measured by the simple determination of weight and height.

The failure to observe clear-cut physical benefits from lunch programs is a familiar experience to workers in the United States[15], but similar negative results in groups of children as apparently retarded as those in the present study were unexpected. Despite the apparent lack of growth response to the lunches supplied, there appeared to be an improvment in rate of gain in height in children receiving

vitamin B_{12}. This response did not quite reach the 5% level of significance.

Studies in Guatemala

Status of the Children Studied

The children of rural Guatemala in the 7 to 11 age range were found to be more retarded in height and weight than those of El Salvador, averaging 2 to 4 years behind U. S. standards. The average retardation in their bone ages was 2.5 years. Clinical findings were essentially the same as in El Salvador including the xerosis and frequency of follicular hyperkeratosis. No signs of scurvy or rickets were encountered. The discrepancy between apparent age and chronological age was somewhat more pronounced in Guatemala. Most of the children were racially and culturally Mayan Indian and 89% belonged to blood group O.

Hematological values were not abnormal. Serum total protein, riboflavin, vitamin C, vitamin E and alkaline phosphatase values were within normal limits[14]. Although the average serum carotene value of 121 micrograms per cent is higher than reported for El Salvador, the level of vitamin A, 27.2 micrograms per cent, is only slightly higher.

Although Necator americanus was not detected in these children, 84% were found to have Ascaris lumbricoides and 36%, Trichiurus trichiura. The children were treated at least once a year for intestinal parasites. All were living at altitudes between 5,000 and 6,000 feet, in a year round climate comparable to spring in the northern United States.

Experimental Design

Four comparable Indian communities near Antigua, Guatemala, and within an hour's drive of Guatemala City were selected for study.

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villages average 7 grams daily. The supplementary snack in the village receiving animal protein, Santa María, provided approximately 8 grams of animal protein, 14 grams of total protein, 350 grams of calcium, 350 units of vitamin A activity and 0.5 mgs. of riboflavin. The supplementary snack in the village receiving vegetable protein, Magdalena, provided approximately 14 grams of total protein, 200 grams of calcium, 400 units of vitamin A activity and 0.5 mgs. of riboflavin. Thus the supplementary snacks greatly improved the situation in regard to all four nutrients, although the vitamin A activity of the total diets was still not optimal.

Results

Table II

Adjusted Gain in Height and Weight of School Children in Guatemala

School	Treatment	Starting Date	No Children	Interval in Months	Average Monthly Gain Height Cms	Average Monthly Gain Weight Kgs
San Antonio	Control	VI-1950	43	11 4	.33	.17
Santa María	Animal Protein Snack	V-1950	81	23.5	.38	.21
Magdalena	Veg. Protein Snack	III-1950	83	28.2	.39	.18
Magdalena	Veg. Protein Snack + B_{12}	III-1950	28	25.3	.43	.24
Magdalena	Veg. Prot Snack + *Aureomycin*	III-1950	29	21.7	.42	.27

Approx L. S. $D._{05}$ for HT = .14 Approx. L. S. $D._{05}$ for WT = .10

In Table 2, the average monthly gains in height and weight for groups starting in 1950 and 1951 are pooled in order to give the largest possible number of cases in each category. The original control group in San Antonio

No attempt was made to substitute a meal for any of the food received by the child at home but rather to supplement the generally skimpy breakfast with a midmorning snack supplying the nutrients considered most likely to be limiting growth. Children in one locality (Santa María) began in 1950 to receive a daily mid-morning snack based on reconstituted powdered dried skim milk provided by UNICEF while those in another (Magdalena) received a similar snack based on the enriched soya milk powder. All of the school children in this latter town were divided randomly into three groups which received in addition capsules containing 50 mgs. of Aureomycin, tablets containing 20 micrograms of vitamin B_{12} or placebos. In the third town, San Antonio, children in a small parochial school and in one of its two public schools constituted control groups and were given placebos. These were considered as one control group in the present analysis. In the fourth town, Xenacoj, the same quantities of vitamin B_{12} and Aureomycin were administered without other supplement. Programs were planned for administration of supplements 6 days a week but were interrupted by special holidays, and vacations from November through January. New groups of children also entered the other schools in subsequent years and were incorporated into the study. Children participating for less than half the possible number of days in any period considered were not included in the tabulations. The number in each original group decreased with each year of the study due to children leaving school or in rare cases leaving the village.

The average diets in the four villages were very similar to those for rural El Salvador, except that the children receiving the vegetable protein supplement in Magdalena proved to have a higher average daily intake of animal protein, 13 grams. Those in the other three Guatemalan

certain political difficulties in the town during 1951, the rates of gain in both height and weight exceed not only those of the children in the original control group in San Antonio but also of the children receiving the animal and vegetable protein snacks in Santa María and Magdalena.

Publication of these seemingly conclusive positive results with vitamin B_{12} and Aureomycin has been delayed for several reasons. Since the control group in San Antonio was made up of children in an entirely different locality, there remained the possibility that environmental factors were producing higher growth rates in all of the children in the town of Xenacoj quite independently of the experimental treatment.

The figures for the twelve months from September of 1951 to September of 1952 give support to the conclusion that vitamin B_{12} and Aureomycin did have a positive effect on height gains in Xenacoj. During this period the INCAP workers were completely withdrawn from the town and no attention or treatment was given to any of the groups. The rate of gain in height of both the former vitamin B_{12} and the former Aureomycin groups dropped. In the former Aureomycin group this drop was sharp and highly significant for both rate of gain in weight and height. In contrast, the rate of gain in weight appeared to increase in the former B_{12} group.

In Santa María, the town in which the animal protein snack was administered, the group in 1950 grew at a rate even lower than the control group in San Antonio, despite the supplementary food provided. In the subsequent two years, the rates of increase in height and weight of this group increased significantly. The groups beginning in 1951 and 1952 did not demonstrate such an initial period of poor response.

showed an average monthly gain of 0.17 kilos in weight and 0.33 cms. in height for 43 children. The 63 children who received the vegetable protein in Magdalena and the 81 who received the animal protein snack in Santa María showed greater rates of gain in both weight and height than this control group. However, no differences were observed between the groups supplemented with the vegetable and animal protein snacks. The addition of vitamin B_{12} or Aureomycin to the vegetable protein snack in Magdalena appears to have had little if any effect on the rate of gain in height. However, the rates of gain in weight do appear to have been influenced somewhat by vitamin B_{12} and by Aureomycin. Unfortunately the variation of individual measurements is such that these trends may be chance results of sampling.

Table III

Supplementation of Current Guatemalan Diets with B_{12} and Aureomycin

School	Treatment	No Children	Interval in Months	Average Monthly Gain Height Cms	Average Monthly Gain Weight Kgs
	MAY 1950-SEPT. 1951				
XENACOJ	B_{12}	16	15	.44	.23
XENACOJ	Aureomycin	14	15	.48	.26
	SEPT 1951-SEPT. 1952				
XENACOJ	B_{12}	12	12	.39	.27
XENACOJ	Aureomycin	11	12	.31	.16

Approx. L. S. $D._{05}$ for HT = 11 Approx. L. S $D._{05}$ for WT = .08

The results of the administration of vitamin B_{12} alone and Aureomycin alone in Xenacoj are shown in Table 3. Although the average attendance was only 63 per cent of the total possible for the vitamin B_{12} groups and 71 per cent for the children receiving Aureomycin, due largely to

children. In fact, it was as great or greater in this special Xenacoj group than was observed in any of the Guatemalan groups studied. This high rate of weight gain in a group of children in Xenacoj receiving no treatment except the placebo capsules during the first half of the observation period, suggests that considerable caution must be used in interpreting weight gain differences among towns.

We should like to believe that under the conditions of the study in Guatemala, the vegetable and animal protein snacks were equally effective. The size of the least significant differences and the divergent weight gain of the special group in Xenacoj warn that such a definite conclusion must be deferred until further data are available. It is tempting to dismiss the failure of vitamin B_{12} and Aureomycin to produce a more definite increase in the rate of gain in height, when given in addition to the vegetable protein snack, as due to the fact that the children were already responding maximally to the basic supplement. If so, would this be for or against the concept of a human *animal protein factor requirement?* The animal protein content of the basic diet in Magdalena was found to be higher than in the other towns. It is possible that the average of approximately 13 grams daily was a sufficient quantity of animal protein to permit a near maximal growth response without added vitamin B_{12}, when other needed nutrients were supplied.

Individual Growth Responses

Occasional individuals showing highly significant increases above the average growth performance occurred in all groups. They contribute disproportionately to the average and standard deviation of the data and have proved a major complicating factor in the drawing of specific con-

Comments

The revisits of the nutritionists to the families in Xenacoj after the B_{12} and Aureomycin had been administered for several months gave some indication that the appetites of at least some of the children receiving these supplements had increased. This may help to explain the apparent growth response even though no food supplement was given.

Whether the initial poor response in Santa María to the animal protein snack was partly associated with the widespread distrust of milk as a food in this town and the conviction on the part of many of the children and their parents that they were having digestive difficulties caused by the milk cannot be stated with certainty. The report of Widdowson[16] describing the reversal of expected effects from supplementary feeding in two German orphanages due to the adverse psychological influence of a harsh superintendent clearly demonstrates the possible importance of such factors in the response to supplementary feeding programs.

In the second year of the study, a relatively large group of new children entered the Xenacoj school most of whom should have been enrolled previously. While thus not strictly comparable to children in the groups originally selected for study, their growth was observed. They were expected to furnish valuable supplementary information on the performance of control groups. When the observations on 70 of these children during the years 1951 and 1952 were tabulated on the same basis as those in tables 2 and 3, the rate of height gain was found to be .32. This is the same as that observed in the San Antonio control group. The rate of weight gain, however, was found to be .27, almost twice that observed for San Antonio

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Occasional individuals showing highly significant increases above the average growth performance occurred in all groups. They contribute disproportionately to the average and standard deviation of the data and have proved a major complicating factor in the drawing of specific con-

clusions by statistical evaluation of the data. Obviously, a great deal remains to be done in the analysis of our records on an individual rather than an average basis. However, it is clear that the selection of *any* group for *any* kind of treatment would include a number of individuals who would respond spectacularly. For evaluations of this type to be based on the number of individuals showing clearly positive growth responses, a control group of equal size and equivalent initial status, receiving some kind of comparable placebo treatment would seem to be absolutely essential.

Discussion

In this initial presentation of the results it is obviously impossible to enter upon a detailed analysis of the multiple factors involved. It has seemed more important to give as accurate a picture as possible of the various trends observed. In doing so we have mentioned data which seem divergent and for which we have no satisfactory explanation, as well as results which seem to help in answering the fundamental questions which the project was designed to study.

When control and experimental groups can be drawn randomly from children in the same school and all groups can receive apparently equal treatment, the results of a single trial can be viewed with considerable confidence. Unfortunately comparisons of animal and vegetable protein cannot be set up in this way. It seems obvious that when a study cannot be conducted in this ideal fashion, the differences in growth rates between control and experimental groups in any single trial, even though appearing to be significant, may be due to factors other than the treatment administered.

Unless a study has true controls, positive conclusions seem to us to be justified only when repeated trials com-

bining different groups give results in the same direction. By this criterion part of the data presented in this report, while highly suggestive, cannot be regarded as conclusive until additional studies are carried out. Nevertheless, the data presented provide grounds for reasonable optimism concerning the potentially positive effects of vitamin B_{12} and Aureomycin on growth under the type of conditions prevailing in rural Central America. If the positive effect of these agents is confirmed by further studies under similar conditions, serious consideration must be given to providing the minimum quantity of animal protein necessary for satisfactory growth in underdeveloped areas or possibly the enrichment of vegetable diets by vitamin B_{12} in some other manner.

Summary

School children 7 to 11 years of age in El Salvador and Guatemala in the lower and lower middle economic levels, were found to be two to four years behind U. S. children in height, weight and bone age. The diets were generally low in animal protein, vitamin A, riboflavin and calcium. The number of children analyzed in the experimental groups varied from 12 to 81.

Under the experimental conditions prevailing in El Salvador, no significant improvement in the rates of gain in height and weight over a three year period resulted from the administration of a lunch rich in animal protein to the children in one rural and one urban school. A similar lunch in one rural school containing only protein from vegetable sources also produced no change in these rates. The addition of 20 micrograms daily of vitamin B_{12} produced no effect on weight but a distinct increase in height which, however, did not reach the 5% level of significance. Children receiving placebos in one urban and one rural school served

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as control. There was doubt regarding the adequacy of the vitamin A intake even in the groups receiving the experimental lunch.

In Guatemala, animal and vegetable protein containing snacks were offered during two and a half years to school children in two rural villages and placebos were administered to children in a third village. Increases in the rates of gain of height were observed but the variations were such that this could have been the chance result of sampling.

One-third of the children in the village receiving vegetable protein were given in addition 20 micrograms of vitamin B_{12} and one-third 50 mgs. of Aureomycin. These supplements brought an apparent increase in the rate of gain in weight but had only a very slight effect on the rate of gain in height. None of the differences reached the 5% level of significance.

Children in one rural Guatemala village were given either 20 micrograms of vitamin B_{12} or 50 mgs. of Aureomycin without other supplementation. Their rates of increase in both height and weight over an 18 month period proved to be significantly greater than those observed for the control group in another rural village. All treatment and attention was suspended for the succeeding twelve months during which the rates of gain in height and weight of the children formerly receiving Aureomycin dropped significantly. The rate of gain in height of the group formerly receiving vitamin B_{12} also dropped although less markedly, but its rate of gain in weight increased. A separate group of slightly older children in Xenacoj receiving no supplement and studied during the last two years of the project showed a rate of gain in height similar to that of the original control children, but a rate of gain in weight as high or higher than that of any other group.

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VITAMIN B_{12} AS A GROWTH FACTOR IN ITALIAN CHILDREN ON DIETS LOW IN ANIMAL PROTEIN*

NORMAN JOLLIFFE, ROBERTO FUNARO, GINO FRONTALI
GIORGIO MAGGIONI, SALVATORE CORBO AND
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Since 1945, we in the nutrition clinics have had as one of our major problems that of "simple growth retardation" in children. By the term "simple growth retardation" we mean underweight in children due to a calorie debt, which may or may not include a protein debt, not caused by organic disease, endocrinopathies and deficiencies of presently recognized essential nutrients. The recognition and treatment of children with these conditions are either well known or not of immediate concern to this study. On the other hand the treatment of "simple growth retardation" is of considerable concern to parents, school authorities and to many professional people. This is true even though we are not always sure that "simple growth retardation" represents necessarily a harmful condition other than the psychological handicap imposed by being different from the majority.

With these factors in mind a two year study was undertaken on 200 children with "simple growth retardation" between the ages of 7 and 11 years who were all subjected to intensive dietary education and all were given supplementary amounts of vitamins A and D. One-half of the children belonged to families whose food supply was sup-

* Supported by a grant-in-aid from The National Vitamin Foundation, Incorporated.

furnish the vitamin B_{12} tablets and placebos. Accordingly, Dr. Funaro went to Italy and during the summer and fall of 1951 together with Professor Frontali and his group, outlined and planned the details of the study and selected an orphanage in Rome, the Asilo Savoia, and an elementary school in Gaeta as the site of the study. The observations throughout the school year were made by the Italian group and then tabulated and analyzed by both groups in the summer and fall of 1952.

The period of observation and administration of the tablets of vitamin B_{12} and the placebos was fixed from November to June. This plan has allowed us to follow for a period of 7 months two groups of children: (a) boys and girls 6 to 10 years of age living in an orphanage on a diet of known composition for the group, and (b) boys and girls 6 to 12 years of age living at home, of various economic categories, but in a depressed area, where the diets were known to be poor. This was checked by diet history obtained on each subject twice during the 7-month period.

All the children at the beginning of the study were subjected to a thorough medical examination with special emphasis on signs of deficiency diseases and which included a tuberculin patch test, X-ray of the chest in the Asilo Savoia, and fluoroscopic examination of the chest in Gaeta. The heights, weights, and chest measurements were made by one of us, the heights and weights at monthly intervals, and the chest measurements in November, March, and May. This has permitted recording of the results on the Correnti Auxogram[2] in November, March, and May. In addition records of absenteeism, illnesses and academic progress were kept. At both locations the children were grouped by class, age, and sex into two homogeneous groups.

plemented by one-half pound of enriched white bread daily for each member of the family. Equally good weight gains were obtained in both groups with about half of the children reaching a weight for height sufficient to be considered normal. A second study was then undertaken in which one group received a multiple vitamin capsule containing supplementary amounts of vitamins A, D, and C, and thiamine, niacinamide and riboflavin. The control group received only supplementary amounts of vitamins A and D. Again the weight gains over the next two years were similar in the two groups.

It was at this time (1949) that vitamin B_{12} became available for oral administration. Also there appeared the preliminary paper of Wetzel, Fargo, Smith and Helikon[1] who reported positive effects as demonstrated by the Wetzel Grid Technique in 5 of 11 children in "simple growth failure." It seemed to us that vitamin B_{12} might influence our children with "simple growth retardation" who had failed to respond by dietary education and to vitamin supplementation with the presently recognized "essential" vitamins. Even though we have had changes in the channel of the Wetzel grid we have not been able to determine how many represent responses to vitamin B_{12} or how many represent chance or a delayed response to dietary education.

It was at this point in the spring of 1951 that Dr. Gino Frontali, Professor of Pediatrics at the University of Rome and Director of the National Center for the Study of Children's Alimentation in Italy, offered through Dr. Roberto Funaro to make a cooperative study with us on the effects of vitamin B_{12} on underweight children on low protein diets in Italy. The National Vitamin Foundation agreed to support this study if final and definitive arrangements could be made and Merck and Company agreed to

furnish the vitamin B_{12} tablets and placebos. Accordingly, Dr. Funaro went to Italy and during the summer and fall of 1951 together with Professor Frontali and his group, outlined and planned the details of the study and selected an orphanage in Rome, the Asilo Savoia, and an elementary school in Gaeta as the site of the study. The observations throughout the school year were made by the Italian group and then tabulated and analyzed by both groups in the summer and fall of 1952.

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To one group were administered 20 micrograms of vitamin B_{12}. To the other were given the placebo tablets identical in appearance to the B_{12} tablets. At the Asilo Savoia the tablets were administered daily except Sunday by the sisters. At the elementary school the tablets were administered on all 6 school days, holidays excepted, by two sanitary assistants. Neither the sisters nor the sanitary assistants knew that one of the tablets was a placebo but they did know that we were testing which of the tablets was better.

Results

At the Asilo Savoia

Here direct control over the food, the central kitchen, and the common table allowed a group dietary analysis. In Table I are given the daily averages for a typical week compared with Frontali-Bacchetta findings in post-war Central Italy and with the U. S. National Research Council's Recommended Daily Allowances for children 7 to 12 years of age. The average total daily calories of 1826 is 20 per cent below the Recommended Daily Allowances and 10 per cent below the average value for children in Central Italy. It is more comparable with the 1775 average daily calories of children of the white collar workers in Italy. It is this group in Italy that has the lowest calorie intake.

Table I

Calorie and Protein Values in the Diet of Children of the Asilo Savoia

In Comparison with the Italian Children's Diet and with the R.D.A.

	Asilo Savoia	Average Central Italy	R.D A.
Total Calories	1825	2033	2250
Total Protein	69 gm.	79.2 gm.	65 gm.
Animal Protein	27 gm.	29.9 gm.	32.5 gm.

The total protein intake of 69 grams is considerably below the 79 grams consumed by children in Central Italy but it is not significantly different from the average of the Recommended Daily Allowance of 65 grams for children 7-12 years of age. The amount of animal protein however is only 27 grams as compared to about 30 grams in Central Italy and the 32.5 grams of the Recommended Daily Allowances.

It thus seems that the diet in the Asilo Savoia is definitely less in calories and in animal protein than the Recommended Daily Allowances and about the same as the lowest economic group in Central Italy. The total protein intake however equals the Recommended Daily Allowances.

Changes in Weight

At the end of the period of observation in June, 1952, with the unavoidable losses due to illness or to transfer of certain children to other institutions or to foster homes there remained a total of 159 under observation, divided as follows: boys receiving vitamin B_{12}—44; boys receiving placebos—44; girls receiving vitamin B_{12}—37; girls receiving placebos—34. Tables II and III indicate that the two groups of boys and girls were relatively homogeneous in age distribution. They were also homogeneous as to initial weights. The average initial weight of the treated boys was 23.2 Kg. $\pm$ 3.23 against 23.8 Kg. $\pm$ 3.22 in the controls. The average initial weight of the vitamin B_{12} treated girls was 24.9 Kg. $\pm$ 4.89 against 24.9 Kg. $\pm$ 4.36 in the controls. Also in Tables II, III and IV are given the average weights for age groups in November and June along with the absolute gain in weight. Both boys and girls, treated and control alike, gained weight over the previous November, but in every age group for both boys and girls the gain in weight for the vitamin B_{12}

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Table III

Asilo Savoia—Girls

Age Years	Number of Treated Children	Average Weight In November	Average Weight In June	Gain in Weight in 7 Months
Treated with vitamin B_{12} (Initial Weight 24.9 ± 4.898).				
6	6	18.700	20.300	+1600
7	8	23.187	24.824	+1637
8	8	24.150	25.587	+1437
9	7	26.442	28.399	+1957
10	8	30.737	33.362	+2625
Total	37	Average Total Gain		1862
Controls (Initial Weight 24.9 ± 4.361).				
6	8	20.130	21.455	+1325
7	3	22.300	22.966	+ 666
8	7	24.180	25.508	+1328
9	7	25.371	26.713	+1342
10	9	30.133	32.310	+2177
Total	34	Average Total Gain		1497

Table IV

Asilo Savoia

Difference of Gain in the Treated Group
In Comparison with the Control Group

BOYS

Age	
6	+250
7	+374
8	+163
9-10	+442
Total Average Gain	+341

group was greater than in the placebo treated group. This fact in itself is very significant in spite of the fact that there are too few in each age group to be statistically significant. The entire group of boys treated with vitamin B_{12} gained on the average 341 grams more than did the placebo treated boys. The entire group of vitamin B_{12} treated girls gained on the average 365 grams more than did the placebo treated girls. The statistical significance of these two groups is indicated by a p slightly greater than 0.05 in the girls and a p slightly less than 0.05 in the boys.

Table II

Asilo Savoia—Boys

Age Years	Number of Treated Children	Average Weight In November	Average Weight In June	Gain in Weight in 7 Months
Treated with vitamin B_{12} (Initial Wt. 23.2 ± 3.23).				
6	2	19.100	20.150	+1050
7	18	21.288	22.915	+1627
8	11	24.672	26.435	+1763
9–10	13	25.546	27.353	+1807
Total	44	Average Total Gain		1688
Controls (Initial Weight 23.8 ± 3 22).				
6	4	19.850	20.600	+ 800
7	15	21.700	22.953	+1253
8	13	25 069	26.669	+1600
9–10	12	26.508	27.883	+1365
Total	44	Average Total Gain		1347

the females than in the males. The intake of animal protein in both boys and girls is clearly inferior to that of the Recommended Daily Allowances being about half in the boys and about two-thirds in the girls.

Table V

Calorie and Protein Values in the Diet of Children of the School of Gaeta
In Comparison with the Average Italian Children's Diet and with the R.D.A.

	School of Gaeta Boys	School of Gaeta Girls	Average Southern Italy	R.D.A.
Total Calories	1772	1573	1764	2250
Total Proteins	62.08 gm.	59.55 gm.	72.90 gm.	65 gm.
Animal Proteins	17.32 gm.	24.25 gm.	22.40 gm.	32.5 gm.

Changes in Weight

At the end of the period of observation in June, 1952 there remained a total of 199 children under observation as follows: boys receiving vitamin B_{12}—52; boys receiving placebos—58; girls receiving vitamin B_{12}—37; girls receiving placebos—52. Tables VI and VII indicate that the age grouping, particularly for the girls, is not as homogeneous as at the Asilo Savoia. In both boys and girls there were more control subjects than treated subjects. The average initial weights of the vitamin B_{12} treated boys were 28.5 Kg. ± 4.64 against 26.9 Kg. ± 3.81 in the controls. The average initial weights of the vitamin B_{12} treated girls were 24.5 Kg. ± 3.24 against 24.8 Kg. ± 3.08 in the controls. Thus the homogenity of the groups in the Gaeta elementary school was not as great as in the Asilo Savoia. In Tables VI, VII and VIII are given the average weights for age in November and June along with the absolute gain in weights. As in the Asilo Savoia, both boys and girls,

GIRLS

Age	
6	+275
7	+973
8	+109
9	+615
10	+448
Total Average Gain	+365

Changes in Height

The absolute and percentage increases in height and changes in the Correnti Auxogram for the boys and girls *in both vitamin B_{12} and placebo groups, age by age, has* been computed and found not to be indicative of any differences.

At the Gaeta Elementary School

The dietary study of the school children who are the subjects of this study will be reported upon in detail at a later time by Dr. Corbo. In Table V are compared the average daily intake of our subjects, male and female, with the Frontali-Bacchetta findings in post-war Southern Italy and with the National Research Council's Recommended Daily Allowances. The total calories of the boys are almost identical to the average in Southern Italy and about 20 per cent lower than the Recommended Daily Allowances. The girls however are 30 per cent lower than the Recommended Daily Allowances and about 10 per cent lower than the average for Southern Italy. Total proteins are significantly lower in both males and females than the average for Southern Italy and somewhat lower than the Recommended Daily Allowances. This difference is greater in

the females than in the males. The intake of animal protein in both boys and girls is clearly inferior to that of the Recommended Daily Allowances being about half in the boys and about two-thirds in the girls.

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treated and control alike, gained weight in June over the preceding November. In the boys the vitamin B_{12} group gained more weight in each age group than did the control group except for the 9-year olds where a 57 grams difference in favor of the control group was noted. In the girls the vitamin B_{12} treated group gained more weight at every age except the 7 and 8 year olds where the numbers in these two age categories are very dissimilar. Statistically none of the results is significant for each age-sex group as the numbers are too small. The entire group of boys in Gaeta treated with B_{12} gained 497 grams more than the control group of boys. This difference has a p slightly less than 0.05. The entire group of girls in Gaeta treated with vitamin B_{12} gained 219 grams more than the control girls. This difference has a p greater than 0.05.

Table VI

School of Gaeta—Boys

Age Years	Number of Treated Children	Average Weight In November	Average Weight In June	Gain in Weight in 7 Months
Treated with vitamin B_{12} (Initial Wt. 28.5 ± 4.643).				
8	15	26.213	28.179	+1967
9	14	27.650	28.992	+1343
10	12	29.383	30.700	+1317
11	8	33.337	35.662	+2325
12	3	28.033	29.700	+1667
Total	52	Average Total Gain		+1677
Controls (Initial Weight 26.9 ± 3.81).				
8	20	25.295	26.445	+1150
9	19	26.742	28.142	+1400
10	10	28.110	28.150	+1040
11	6	26.825	27.752	+ 967
12	3	33.210	33.967	+ 757
Total	58	Average Total Gain		+1180

Table VII

School of Gaeta—Girls

Age Years	Number of Treated Children	Average Weight In November	Average Weight In June	Gain in Weight in 7 Months
Treated with vitamin B_{12} (Initial weight 24.5 ± 3.238).				
7	13	22.784	24.214	+1430
8	3	20.666	21.976	+1300
9	12	25.825	27.558	+1733
10	7	25.414	27.157	+1743
11	2	30.700	32.100	+1400
Total	37	Average Total Gain		+1575
Controls (Initial weight 24.8 ± 3.082).				
7	4	23.675	25.225	+1500
8	11	22.963	24.399	+1436
9	16	24.981	26.224	+1243
10	17	25.155	26.558	+1403
11	4	28.450	29.400	+ 950
Total	52	Average Total Gain		+1356

Table VIII

Difference of Gain in the Treated Group
In Comparison with the Control Group

School of Gaeta

BOYS

Age	
8	+817
9	— 57
10	+277
11	+1358
12	+910
Total Average Gain	+497

treated and control alike, gained weight in June over the preceding November. In the boys the vitamin B_{12} group gained more weight in each age group than did the control group except for the 9-year olds where a 57 grams difference in favor of the control group was noted. In the girls the vitamin B_{12} treated group gained more weight at every age except the 7 and 8 year olds where the numbers in these two age categories are very dissimilar. Statistically none of the results is significant for each age-sex group as the numbers are too small. The entire group of boys in Gaeta treated with B_{12} gained 497 grams more than the control group of boys. This difference has a p slightly less than 0.05. The entire group of girls in Gaeta treated with vitamin B_{12} gained 219 grams more than the control girls. This difference has a p greater than 0.05.

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11	6	26 825	27.752	+ 967
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Total	58	Average Total Gain		+1180

the method of covariance analysis of the combined data the results indicate that the greater gain in weight in June of the vitamin B_{12} treated group had a p of 0.001. Hence the greater gain in weight in favor of the B_{12} treated children may be considered highly significant.

Summary

A group of 351 children consisting of boys between *the* ages of 6 and 12 and girls between the ages of 6 and 11 residing either in an orphanage (Asilo Savoia) in Rome or living in a depressed area and attending an elementary school at Gaeta who were subsisting on a diet containing 20 to 30 per cent less calories than the Recommended Daily Allowances and 15 to 40 per cent less animal protein than the Recommended Daily Allowances were divided into two approximately similar groups one of whom received 20 micrograms of vitamin B_{12} daily by mouth and the other a placebo tablet of similar appearance. The 172 who received vitamin B_{12} made an average gain of 345 grams more than the group of 179 who received the placebo tablets. This increased gain in weight is highly significant with a probability of it occurring by chance being only 1 in 1000. When these results are divided into boys and girls residing at the Asilo Savoia or in Gaeta and compared with the same groups receiving placebos, each group receiving B_{12} made better weight gains than the placebo group. In 2 of the groups the results are of low order of significance and in 2 groups they probably are not significant. Further subdivision into age groups are statistically not significant.

We found no differences in gains in heights in the two groups.

GIRLS

Age	
7	— 70
8	—136
9	+490
10	+340
11	+450
Total Average Gain	+219

Changes in Height

As in the Asilo Savoia the absolute and percentage increase in height and changes in the Correnti Auxogram for the boys and girls in both the vitamin B_{12} and control groups, age by age, has been computed and found not to be indicative of any difference.

Combined Results of Asilo Savoia and the Elementary School at Gaeta

Since both boys and girls at both localities receiving vitamin B_{12} made a greater gain in weight than did their companions receiving placebo tablets, and since in 2 of these groups the degree of significance equalled a p greater than 0.05 and in 2 groups the p was slightly less than 0.05 it seemed likely that had we larger numbers in each of these groups the results would be statistically significant. Since we included children in comparable age groups, boys 6 to 12, and girls 6 to 11, in which sex plays no particular role as far as weight for age and height is concerned and since in both groups the pre-puberty spurt is largely eliminated we feel it is justified to combine the boys and girls at both Asilo Savoia and at Gaeta into two groups, those treated with B_{12} and those given placebos. When this is done by

THE SIGNIFICANCE OF NUTRITION AND NUTRITIONAL DEFICIENCIES IN PREGNANCY#*

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It is questionable if the adult human female is ever subjected to a greater stress than during pregnancy; and it is believed that pregnancy represents a degree, intensity and velocity of stress which has been wholly minimized by its acceptance as a normal physiologic process. Unquestionably, pregnancy should be a normal physiologic and metabolic process, but rarely if ever, do we see a patient whose status at the beginning of pregnancy is optimum. The constant stresses throughout life, for which complete compensation has rarely occurred result in some level of dysfunction. These are not necessarily levels of absolute or complete deficiency, but levels which leave a margin of safety which may not be commensurate with sudden and prolonged stress should it be applied.

Objective Evidence of Deficiency

We feel that the objective evidences of deficiency have not been properly recognized as a critical and necessary part of the evaluation of the pregnant patient. Although nutritional deficiency of an advanced degree is comparatively uncommon in most areas, the less severe states are

The Nutrition Research Clinic is supported by Grants-in-aid from the Milbank Memorial Fund, the National Vitamin Foundation, the Williams-Waterman Fund, the Nutrition Foundation, the Upjohn Company, Mead Johnson and Company and E. R. Squibb & Sons.

* The material and data contained in chapter 9 of "Clinical Obstetrics", published by J. B. Lippincott and Co., Philadelphia, (1953), has been drawn upon liberally in the preparation of this presentation.

The period of observation, 7 months, was too short for valid analysis of the data by Correnti's Auxogram. Observations are being continued for an additional year.

We conclude that under the conditions of our study the daily oral administration of 20 micrograms of vitamin B_{12} resulted in a highly significant increase in weight gain for a period of 7 months in children between the ages of 7 and 11 whose diets were lower in calories and in animal protein than that of the Recommended Daily Allowances.

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the therapeutic procedures which were instituted during and after these episodes occurred.

It is of little clinical importance as to whether or not these changes are the result of a specific nutrient lack; the fact that these tissues change due to the stress of pregnancy is important, and that they can be protected or will respond in a favorable direction, following adequate nutritional therapy, is more important.

Chemistry

Biochemical changes incident to pregnancy are many but rarely are they demonstrable in a significant manner until other evidences such as can be observed clinically have already been established. It is apparent that changes in body fluid levels commence very soon after the onset of pregnancy. Although these changes in levels occur gradually, they are definite and indicate the wide margin of functional safety inherent in stabilized individuals. Unless actual damage to a specific area precedes the onset of pregnancy, such vital areas as the liver, kidneys and adrenal cortex, for example, have a functional margin of activity commensurate with the stress of pregnancy, and functional tests rarely show marked deviations from the generally established limits of acceptability. However, there is evidence that these so-called "normal" limits have been established on groups of patients that include many who were sub-optimal in status.

Our data suggest that these so-called acceptable limits are too broad, or our presently available techniques are lacking in specificity. Such determinations for example, as total serum protein, simply indicate that amount which is available in the circulation at the time the sample is obtained. It in no way reflects what would be most desirable to know,

by no means uncommon. It is with these less severe states that we are primarily concerned. Being less severe or less acute, they permit insidious development of a negative nutritional balance; whereas the more severe forms would be recognized promptly and treated accordingly. The tissue changes characteristic of deficiency processes are readily observable[1]. These changes can be seen in the tongue, gums and sclera; and are significant in that they indicate the degree of stress to which the individual is being subjected, or the individual's ability to adjust to the physiologic as well as the physical stress of pregnancy. Critical evaluation of the observable tissues gives an indication of the cellular adequacy or depletion in non-observable areas, and suggests the margin of safety available in the individual observed. We feel it is reasonable to assume, that if these tissue areas show observable alterations from optimum at the beginning of pregnancy, and this process continues to progress unfavorably, the pregnancy was the precipitating force in activating these changes. Since it is impossible to have a single deficiency, it must be recognized that evidence of a specific nutrient deficit is evidence of lack of other essential nutrients, or a failure in metabolism and physiology relative to the requirements of the individual.

Unfortunately, perfect or optimum tissue status in observable areas have never been observed in the adult human. The reasons for this result from periodic episodes of stress which have not been adequately compensated for, and we are actually observing the evidence of residual deprivation resulting from these episodes. Thus when these visible tissue areas are evaluated, we are confronted with tissue which has existed in a damaged state for a prolonged period of time. The observable evidence of previous damage will be determined by the severity, frequency and duration of these periods of stress, and the adequacy of

in maintaining total serum protein at adequate levels. We have found this to be of clinical significance in the evaluation of a patient's status in considering the possibility of developing toxemia. Preliminary observations suggest that a failure of globulin production is evidence that the patient is failing to meet the increased requirements of pregnancy by providing an adequate supply of available protein.

Protein Levels and Symptoms of Toxemia

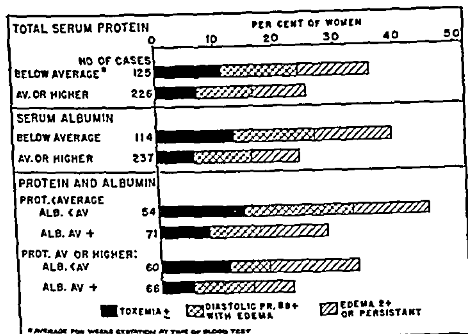


FIGURE 1. *Frequency of symptoms associated with toxemia of pregnancy among 351 women classified according to total serum protein, total serum albumin, and protein and albumin levels.*

Clinically an important question is whether, or not, serum protein levels have any relation to the complications of pregnancy and aid in the prognosis of dysfunctions which may occur. Figure 1 indicates that the frequency of toxemia and symptoms associated with toxemia of pregnancy vary

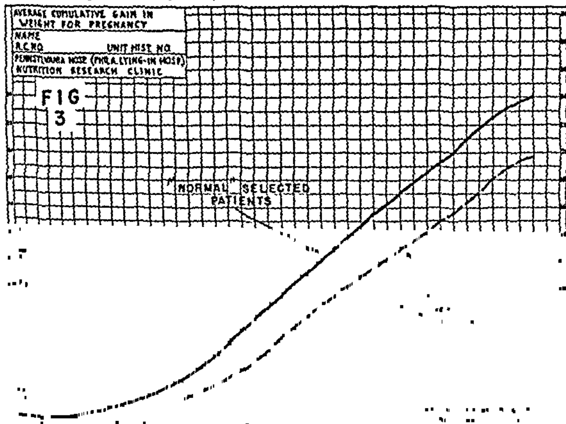
namely, the source of the level or the mechanism by which it was maintained. These levels should be maintained by sufficient intake of dietary essential nutrients and an efficient absorption and utilization mechanism. However when the dietary supply is in deficit or a conditioned deficiency exists, a satisfactory level can be maintained only through disruption of tissue integrity by means of the catabolic mechanism. It is when this catabolic mechanism fails, that the situation becomes critical and an isolated determination, therefore, may not necessarily indicate the true process which is operating within the individual at the time.

Certain changes in blood values demonstrable immediately after the onset of pregnancy give evidence, and are of clinical prognostic value, in evaluating a patient's response to the demands caused by these changes, and can suggest the relative degree of deprivation present.

Wiehl[2] has demonstrated that serum globulin should increase steadily during the 2nd and 3rd trimesters of pregnancy. This increase is sufficient to offset the decrease in albumin after about 20 weeks of gestation and to maintain total serum protein at a fairly constant level during the latter half of pregnancy.

Albumin is needed during pregnancy to build new tissue for the placenta and fetus, to maintain tissue integrity and function within the patient, and it must be withdrawn from the circulation in considerable amounts. The demand for total protein levels that will maintain a satisfactory metabolic and physiologic balance apparently is met by a marked increase in globulin. A recent experiment by Miller[3] on dogs using tagged DL-Lysine showed that plasma globulin was synthesized more readily than albumin. Under the stress of pregnancy, a rapid synthesis of globulin seems to occur; and apparently operates as a protective mechanism

Weight in Pregnancy



One of the most significant changes occurring in pregnancy, the result of alterations in metabolism and physiology, is the change in the patient's weight. Analysis of our data for gains in weight of selected patients resulted in a total average cumulative gain in weight during pregnancy of 24 lbs.[4], Figure 3.

Premature Labor

The problem of prematurity is generally considered to be pediatric rather than obstetric; but obviously without premature labor there would not be a premature baby. Consequently, a new approach must be undertaken, which

with the level of total serum protein and serum albumin. This again demonstrates the significance of the globulin level since a deprivation of albumin must be met by an increased production of globulin to avoid toxemia and toxic symp-

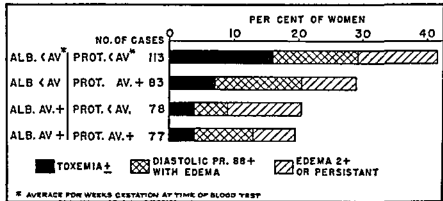


FIGURE 2. *Frequency of symptoms associated with toxemia of pregnancy among 351 women classified in four groups; two groups on basis of lowest serum albumin value relative to average level at a given period of pregnancy and each albumin group subdivided according to total serum protein value.*

toms. Figure 2 also emphasizes the hazard to the patient when a deficiency in protein occurs whether from dietary lack, catabolic failure, or conditioned deprivation. Here again it is evident that when the globulin producing mechanism fails there is a sharp increase in the incidence of toxemia and toxic symptoms.

or above, the incidence of premature labor was essentially the same. Among the patients 5% or more underweight, the incidence of premature labor is *twice* as high as among those of standard weight or above; and is almost *four* times as high among those patients 20% or more underweight. The causal relationship suggested by this striking difference in the rate of premature labor has apparently been overlooked in practically all consideration of this problem.

Weight Change in First Trimester

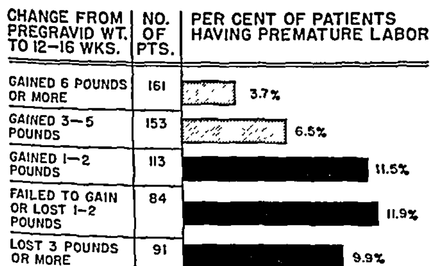


FIGURE 5. *Premature labor according to gain in weight during first trimester.*

There is a significant association between weight loss in the first trimester and the frequency of premature labor. Of particular interest is the fact that the premature labor rate does not decrease until an average gain of *3 lbs. or more* is reached, Figure 5.

will eliminate the causative factors concerned with the early onset of spontaneous premature labor.

Data relative to the patient's weight at the time she becomes pregnant, and her gain in weight during pregnancy, have produced a pattern as indicative of an increased probability of premature labor as was found relative to toxemia. This pattern differs completely, however, from that associated with toxemia, *except* that the individual who is underweight at the time she becomes pregnant, is the *greatest obstetrical hazard, having the greatest probability of both* toxemia and premature labor.

Premature Labor and Immediate Pregravid Weight Status

PREGRAVID WEIGHT STATUS	NO. OF PTS.	PER CENT OF PATIENTS HAVING PREMATURE LABOR
20% OR MORE OVERWEIGHT	99	4%
10–19% OVERWEIGHT	71	5.6%
LESS THAN 10% OVERWEIGHT TO 5% UNDERWEIGHT	264	5.7%
MORE THAN 5% UNDERWEIGHT	220	11.8%
6–19% UNDERWEIGHT	193	10.4%
20% OR MORE UNDERWEIGHT	27	22.2%

FIGURE 4. *Premature labor and pregravid weight.*

In Figure 4 the frequency of premature labor according to immediate pregravid weight status of the patient, demonstrates that among the patients of standard weight

or above, the incidence of premature labor was essentially the same. Among the patients 5% or more underweight, the incidence of premature labor is *twice* as high as among those of standard weight or above; and is almost *four* times as high among those patients 20% or more underweight. The causal relationship suggested by this striking difference in the rate of premature labor has apparently been overlooked in practically all consideration of this problem.

Weight Change in First Trimester

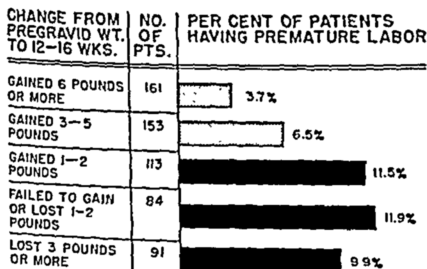


FIGURE 5. *Premature labor according to gain in weight during first trimester.*

There is a significant association between weight loss in the first trimester and the frequency of premature labor. Of particular interest is the fact that the premature labor rate does not decrease until an average gain of *3 lbs. or more* is reached, Figure 5.

Weight Change in Second Trimester

GAIN IN WEIGHT TO 12–16 WKS.	GAIN FROM FIRST VISIT TO 24–26 WKS.	NO. OF PTS.	PER CENT OF PATIENTS HAVING PREMATURE LABOR
AVERAGE GAIN OR MORE	AVERAGE GAIN OR MORE	204	3.9%
	LESS THAN AVERAGE GAIN	110	7.3%
LESS THAN AVERAGE GAIN	AVERAGE GAIN OR MORE	131	6.1%
	LESS THAN AVERAGE GAIN	157	15.3%

FIGURE 6 *Premature labor according to gain in weight during first and during second trimester.*

In Figure 6 the patients are classified according to the weight change in the first two periods of pregnancy. Those who failed to maintain an average or better gain during the second trimester, although their previous gain had been normal, had a premature labor rate 75% higher than those patients with an early normal gain and who continued to gain average or better during the second trimester.

However, the relation of gain during the second trimester to the premature labor rate is shown more strikingly for patients whose gain in the first trimester was less than average.

If the gain during the first trimester was less than average and continued to be less than average during the second trimester, the premature labor rate was 150% higher. Thus, less than average gain in both the first and second trimesters was associated with a premature labor rate two or more times greater than for patients with average gain or better either in the first or second trimester.

Immediate Pregravid Weight and Gain in Weight During First and During Second Trimesters

The premature labor rate is greatly affected by the immediate pregravid weight status of the patient, as well as her pattern of gain during the first and during the second trimesters. When the immediate pregravid weight status and the pattern of gain during the first two trimesters are considered together, the patients who were underweight had twice the frequency of premature labor as did the other patients. Among those patients 5% or more underweight who had less than average gain, or lost weight during the first trimester, and failed to show an average gain in the second trimester, the premature labor rate was 24%.

Weight Change in Third Trimester

The gain in weight during the third trimester does not show a definite association with the occurrence of premature labor.

If remedial procedures are to be instituted in an effort to preclude the onset of premature labor, every effort must be made as early in pregnancy as is possible. The pattern for spontaneous premature labor is established very early in pregnancy, and by the end of the second trimester this pattern cannot be altered.

It should have become obvious long ago that the individual markedly underweight is not obtaining a sufficient quantity of nutrient intake to maintain standard body weight, and is certainly lacking in the essential nutrients necessary for the support of normal body function. It is also rational to assume that since adequate nutrition is necessary for the maintenance of normal body function, successful support of pregnancy will require an added intake of essential nutrients.

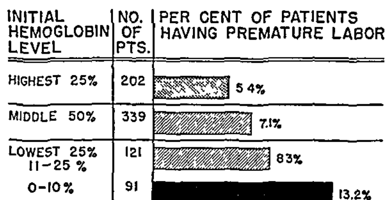


FIGURE 7. *Premature labor according to initial hemoglobin level.*

It is not surprising that anemia, with its attendant anoxia, should have an effect on the incidence of premature labor. Figure 7 classifies patients according to their earliest hemoglobin value; the premature labor rate increasing as the hemoglobin level decreases. This increase in rate however, is not statistically significant until the lowest 10% level is reached, at which point the rate becomes 13% or approximately twice that for patients having an average or higher hemoglobin level.

Premature Labor and Supplementation

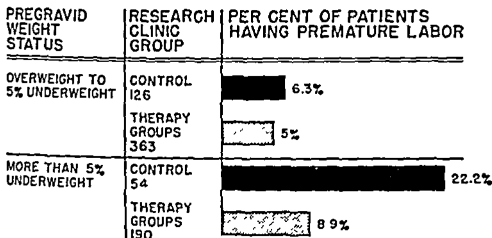


FIGURE 8. *Premature labor according to pregravid weight and supplementation.*

The causal relationship of nutritional deficiencies to premature labor can also be shown by the patient's response to supplemental therapy. Figure 8 considers the frequency of premature labor in relation to immediate pregravid weight among patients in the control and therapy groups. A striking difference was observed between the underweight and overweight patients. Among the patients who were more than 5% below standard weight at the beginning of pregnancy, the control group had a premature labor rate of 22% as against 9% for comparable patients receiving therapy.

Toxemias of Pregnancy

It is generally considered that the term "toxemia of pregnancy" is a misnomer. It is our opinion that the so-called "toxemias of pregnancy" are in reality nutritional deficiency states.

It is well known that the individual definitely *overweight at the beginning*, or who gains *excessively during pregnancy*, has an increased incidence of toxemia. However, consideration has not been given to those individuals who are markedly *underweight;* yet the incidence of toxemia is *twice* as great as compared to those overweight, and nearly seven times greater than those who are standard weight.

Toxemia and Immediate Pregravid Weight

IMMEDIATE PREGRAVID WEIGHT STATUS	NO. OF PTS.	PER CENT OF PATIENTS HAVING TOXEMIA
20% OR MORE OVERWEIGHT	99	6%
+ 19% TO – 19%	528	1.7%
20% OR MORE UNDERWEIGHT	27	11.1%

FIGURE 9. *Toxemia according to immediate pregravid weight status.*

Figure 9 illustrates the significance of three immediate pregravid weight classifications. This table indicates the weight of the patients only at the time they *became* pregnant in relation to the incidence of toxemia, and does *not* consider weight changes occurring during the interval between the beginning of pregnancy and the onset of toxemia. Here is evidence of the critical effect of preexisting deprivation. It is therefore mandatory that deficiency be recognized early in pregnancy and that remedial procedures begin immediately. Any delay in the recognition and treatment seriously increases the hazard to both mother and child.

First Trimester Gain in Weight and Toxemia

There is a definite tendency for the incident of toxemia and toxic symptoms to increase as the rate of gain in weight increases during the first trimester. Marked changes in weight must be considered one of the earliest warnings of dysfunction, even though these early changes are not as significant as changes occurring later in pregnancy.

Second Trimester Gain in Weight and Toxemia

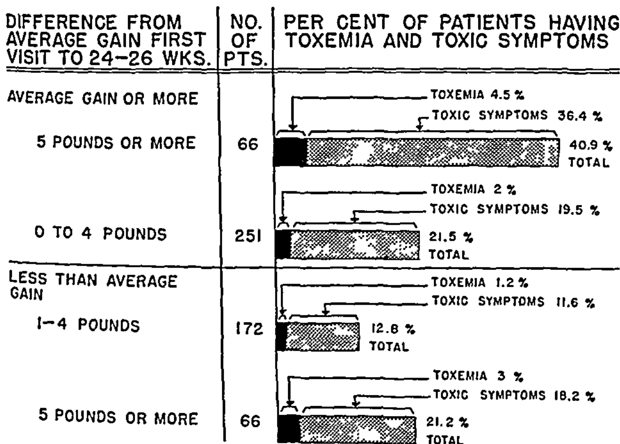


FIGURE 10. *Toxemia and toxic symptoms according to gain in weight during second trimester.*

Changes in weight which occurred during the second trimester were significantly associated with differences in the probability of toxemia or toxic symptoms. It is particularly striking that those patients who gained 5 lbs. or *more above* normal during this period had an incidence nearly *twice the average rate.* Thus variations occurring during the usual prenatal visit interval take on added importance; and must be projected in a cumulative way, to a period later in pregnancy, if the true significance of this change is to be appreciated, Figure 10.

Third Trimester Gain in Weight and Toxemia

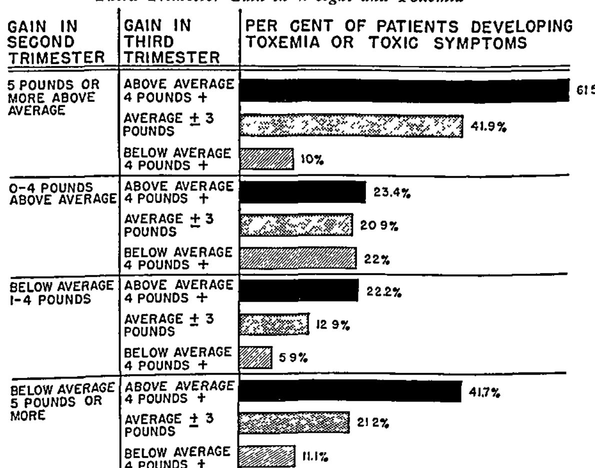


FIGURE 11. *Toxemia or toxic symptoms according to gain in weight during second and during third trimester.*

Variations from an acceptable rate of gain during the first two trimesters become more apparent as the changes in the rate of gain during the third trimester are correlated with the pattern of gain during the first two trimesters. Figure 11 indicates that certain causal relationships can be drawn concerning the association between gain in weight and the incidence of toxic symptoms.

First—for patients who gained excessively up to the end of the second trimester, and *continued* to gain at *more* than the average rate in the third trimester, the incidence of toxic symptoms was about 50%.

Second—the incidence of patients with toxic symptoms was high—42% among the small number of patients who had gained 5 lbs. or more *less* than average by the end of the second trimester, and then gained 4 lbs. or more in *excess* of average during the third trimester; which represents a very marked shift in the rate of gain.

Third—the probability of developing toxemia is, in the majority of cases, determined by the gain which is established by the end of the second trimester.

It is thus apparent that attitudes which disregarded the patient's essential nutrient needs during the first *two* trimesters create a metabolic and physiologic pattern which seriously increases the probability of toxemia.

Toxemia, Toxic Symptoms and Supplementation

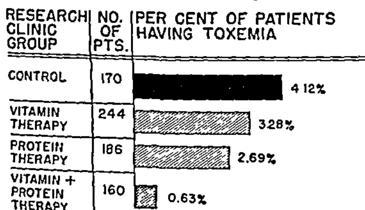


FIGURE 12. *Toxemia and supplementation.*

Evidence of the causal relationship between nutritional deficiency and toxemia can be shown by the patient's response to nutrient supplements. In Figure 12 it is apparent that although the vitamin and protein supplemented groups had a slightly lower percentage of patients developing toxemia, these groups do not differ significantly from the control group. But, among patients who received both vitamin and protein therapy, there was only *one case of pre-eclampsia,* or about 1/6th the rate among patients in the control group.

Weight Changes in Relation to Toxemias for Patients 25% or More Overweight

Since the incidence of toxemia and toxic symptoms is significantly higher among obese patients than among those of approximately normal weight, the changes in weight of these obese patients during pregnancy, and their relation to toxemia and toxic symptoms, have been studied separately from other patients.

With the realization that pregnancy, or any other severe stress, drastically increases the need for essential nutrients, it is difficult to comprehend the procedure proposed by many of arbitrarily restricting these nutrients when the need is most critical.

To justify a marked restriction in the gain in weight, or to actually reduce the weight, of an obese patient during pregnancy *it would have to be established that the initial* nutritional status, nutrient reserve and metabolic efficiency were compatible with such a procedure. The simple reliance on excess fat storage as a source of calories does not guarantee protection to the maternal organism, the placenta or the fetus. The scale indicates only changes in pounds gained

or lost but does not indicate the more essential factor, namely, the quality and character of the nutrient intake associated with these changes. Unfortunately, optimal maternal and infant status are not derived from calories alone, whether of dietary origin or stored excess body fat.

An analysis of our data for gains in weight of normal obese patients resulted in a total average cumulative gain in weight during pregnancy of 19.25 lbs. This cumulative curve, for obese patients, by weeks of gestation is represented by the dotted line in Figure 3 and is contrasted with the average cumulative curve for normal selected patients who were not obese. An average curve for these obese patients for gain during the entire prenatal period was desired that could be considered representative of the gain experienced by those patients who had had the most satisfactory pregnancies. These obese patients had pregnancies which were without evidence of toxic symptoms, toxemia, premature babies, or fetal abnormalities; and all patients were delivered at term uneventfully. Although the ideal, or optimal, gain is not necessarily represented by the average gain for patients without complications and without major signs or symptoms of dysfunction, the average curve for such patients does describe gain in weight that is compatible with a better than average pregnancy in the clinical sense.

During the first trimester, the obese patient gained approximately half the amount of the non-obese patient. During the second trimester the cumulative difference for gain between the obese and non-obese patient is slightly under 4 lbs. less than the average for the non-obese patients. By the end of the third trimester this differential is slightly over 4 lbs.

Weight Changes in First Trimester

Like the non-obese patient, there is no significant variation in the incidence of toxic symptoms for obese patients in relation to weight changes in the first trimester.

Weight Changes During the Second Trimester

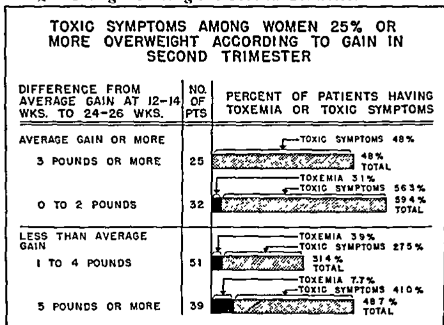


FIGURE 13. *Toxemia and toxic symptoms according to gain in weight during second trimester.*

Table 1

Frequency of Toxemia and Toxic Symptoms Among Obese* Patients According to Gain in Weight During Second Trimester

Difference from Average Gain During Second Trimester		No of Patients	Number of Cases: Toxemia	Number of Cases: Toxic Symptoms	Number of Cases: Total	Per Cent of Cases: Toxemia	Per Cent of Cases: Toxic Symptoms	Per Cent of Cases: Total
	Total	147	5	60	65	3.4	40.8	44.2
Above Average	3 lbs.+	25	0	12	12	0	48.0	48.0
	0 to 2 lbs.	32	1	18	19	3.1	56.3	59.4
Below Average	1 to 4 lbs.	51	1	14	15	2.0	27.4	29.4
	5 lbs.+	39	3	16	19	7.7	41.0	48.7

* Patients 25% or more overweight

Gain in weight during the second trimester, as shown in Figure 13 and Table 1, shows the same relation to the incidence of patients with toxic symptoms that was found for patients who were not obese. That is, the minimum incidence occurred among those whose gain was 1 – 4 lbs. less than average for the observed period. The incidence increased for those having a failure to gain of more than 4 lbs., and the incidence was highest for those who gained in excess of average.

The low incidence (29.4%) of cases with toxic symptoms among patients whose gain was 1 – 4 lbs. below average, compared with an incidence of 59.4% among those who gained average or slightly higher, can not be interpreted as indicating that less than average gain was more favorable to the patients. Patients who had been gaining less than average for part or most of the second trimester, may have an accelerated gain and move into the higher than average gain group if edema develops.

Weight Changes in the Third Trimester

Table 2

Frequency of Toxemia and Toxic Symptoms Among Obese* Patients According to Gain in Weight During Third Trimester

Difference from Average Gain During Third Trimester		No. of Patients	Number of Cases: Toxemia	Number of Cases: Toxic Symptoms	Number of Cases: Total	Per Cent of Cases: Toxemia	Per Cent of Cases: Toxic Symptoms	Per Cent of Cases: Total
	Total	147	5	60	65	3.4	40.8	44.2
Above Average	4 lbs.+	24	1	17	18	4.2	70.8	75.0
	1-3 lbs.	34	2	14	16	5.9	41.2	47.1
Below Average	0-3 lbs.	58	2	18	20	3.4	31.0	34.5
	4 lbs.+	31	0	11	11	0	35.5	35.5

* Patients 25% or more overweight.

For obese patients whose gain in weight in the third trimester was 4 lbs. or more above average for the period, the incidence of toxic symptoms was 75%. For all others the incidence was about 40%, and there was no difference in the incidence according to the amount of weight gained as shown in Table 2.

Table 3

Frequency of Toxemia and Toxic Symptoms Among Obese* Patients According to Gain in Weight During Second and Third Trimester

Difference from Average Gain During Second and Third Trimesters		No of Patients	Number of Cases			Per Cent of Cases		
			Toxemia	Toxic Symptoms	Total	Toxemia	Toxic Symptoms	Total
	Total	147	5	60	65	3.4	40.8	44.2
Above Average	5 lbs. or more	37	1	23	24	2.7	62.1	64.8
	1 to 4 lbs.	28	1	11	12	3.6	39.3	42.8
Below Average	0 to 3 lbs.	32	0	10	10	0	31.2	31.2
	4 to 7 lbs.	21	1	4	5	4.8	19.0	23.8
	8 lbs. or more	29	2	12	14	6.9	41.4	48.3

* Patients 25% or more overweight.

The risk of developing toxemia is high for obese patients. This is best demonstrated by Table 3, which indicates the effect of cumulative gains in excess or below average during the second and third trimesters. Here it should be noted that the obese patient who establishes a pattern of gain which is steadily in excess of average gain has a better than 50% chance of becoming toxic. It is very striking that when the total cumulative gain for the entire second and third trimesters is only 5 lbs. or more, 65% of the obese patients became toxic. The best protection is afforded the obese patient if her gain is average or slightly less than average, but it should be noted that 27.5% of these obese patients still become toxic. Of equal importance is

the fact that when the obese patient's pattern of gain reaches 8 lbs. or more *less* than average for the entire second and third trimester, approximately 50% of these patients become toxic.

We believe there is conclusive evidence from these data that the obese patient is a most critical individual and must be managed accordingly. The radical restrictions in gain in weight for obese patients which have been proposed have been demonstrated to be dangerous, and these patients' weight must not be reduced during pregnancy.

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Includes discussions of nutritional factors related to intestinal absorption, of nutritional aspects of hepatic disease, of pancreatic insufficiency, of nutritional disturbances in ulcerative colitis, of nutrition in tissue devitalization and maintenance, of induced nutritional deficiencies and some international economic aspects of nutrition.

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Proceedings of the Scientific Sessions of the Seventh Annual Meeting of The National Vitamin Foundation, Incorporated, New York City, April 3, 1952. A. E. Axelrod, B. F. Chow, T. J. Cunha, R. L. Davis, R. W. Heinle, W. A. Krehl, R. W. Luecke, E. P. Ralli, W. Schreiner, R. B. Schultz, R. W. Vilter, H. D. Wallace, D. R. Weir and R. W. Winters.

Includes discussions of the metabolic role of vitamin B_{12}, of substances combining with vitamin B_{12}, of the vitamins in antibody production, of the adrenals in pantothenate deficiency, of the effect of nutritional factors on reactions to acute stress in humans, of the B vitamins in leukopoiesis, of pyridoxine deficiency in man and seborrheic dermatitis, of the B vitamins in the nutrition of the pig and of antibiotics in swine rations.

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Proceedings of the Nutrition Symposium held at the Harvard School of Public Health, October 29, 1952. David P. Barr, John R. Brobeck, Henry W. Brosin, Louis I. Dublin, Frank A. Evans, P. C. Fry, Samuel Gurin, Paul György, Edward E. Hunt, Jr., Ancel Keys, P. S. Peckos and A. W. Pennington.

Includes discussions of lipogenesis, of body composition and of the definition of obesity, of the physiology of appetite, of the psychology of overeating, of the effects of obesity on morbidity and mortality, of nutrition in liver disease and recommendations on practical reducing regimens.